Theta Burst Stimulation of Human Cortex

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Abstract

It has proved possible in experimental animals to manipulate synaptic efficiency using direct electrical stimulation of central nervous pathways. The introduction of transcranial methods of non-invasively stimulating the human brain raised hopes that similar effects could be produced in humans, with the potential for eventual therapeutic application in disease states. However, largely due to safety limitations, human subjects often require lengthy conditioning with repetitive transcranial magnetic stimulation (rTMS), and even then effects are often weak, variable and not beneficial in the therapeutic setting. In view of the difficulties, I have explored novel methods of conditioning the brain in conscious humans using rTMS which can swiftly produce powerful and controllable long-term changes in the excitability of cortical circuits.

I have developed novel rTMS paradigms based on theta burst (TBS) patterns of neuronal firing occurring in the hippocampus of animals, which is an efficient way to produce long term potentiation in animal preparations. Very short (20 - 190 seconds) conditioning using different TBS patterns at low intensity (80% of active motor threshold) can safely produce controllable, consistent, long-lasting and powerful LTD and LTP-like effects on the motor system in conscious humans at an electrophysiological and behavioural level that outlast the period of stimulation by over an hour. In particular I have found that the pattern of delivery of TBS (continuous versus intermittent) is crucial in determining the direction of change in synaptic efficiency. I have therefore explored the mechanism of these effects and have found evidence for paired inhibitory and excitatory consequences of TBS that

can be manipulated through the use of different patterns of stimulation. Based on this, I have created a theory together with a theoretical mathematical modelling to explain the mechanism.

Furthermore, I have demonstrated that during and after the repetitive stimulation physiological activity altering the cortical excitability can modulate the effect of plasticity induced by the stimulation. I have also used theta burst conditioning to show that the response to experimental 'plasticity power' is abnormal in DYT1 gene carriers, suggesting that overplastic brain could be the cause of dystonia while the non-manifesting individuals may be protected by reduced plasticity.

In conclusion, I have developed novel methods of delivering rTMS based on patterns of theta burst stimulation, which are controllable, consistent, long-lasting and powerful. I also have explored the underlying mechanism and performed mathematical modelling of the theory. In addition, I have presented nice examples of the application of TBS.

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	active motor threshold
BCM theory	Bienenstock–Cooper–Munro theory
Bic	bicuculline methiodide
BOLD	blood oxygenation level dependent
Ca	Calcium
CaMKII	calcium-CaM-dependent protein kinase II
EEG	electroencephalography
EMG	electromyography
EPSC	excitatory postsynaptic currents
EPSP	excitatory post-synaptic potential
FDI	first dorsal interosseous
FMRFamide	Phe-Met-Arg-Phe-NH_2
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GPCR	G-protein-coupled receptors
HFS	high frequency stimulation
ICF	intracortical facilitation
ICI	intracortical inhibition
iGluR	ionotropic glutamate receptor
$InsP_3$	inositol triphosphate
ISI	inter-stimulus interval
LFS	low frequency stimulation
LICI	long-interval intracortical inhibition

LICR	ligand-gated ion channel receptors
LPMC	lateral premotor cortex
LTD	long-term depression
LTP	long-term potentiation
MEP	motor evoked potential
mGluR	metabotropic glutamate receptor
MRI	magnetic resonance imaging
NMDA	*N*-methyl-D-aspartate
PAS	paired associative stimulation
PET	positron emission tomography
PKA	protein kinase A
PKC	protein kinase C
PSD	postsynaptic density
rCBF	regional cerebral blood flow
RMT	resting motor threshold
rTMS	repetitive transcranial magnetic stimulation
RyRs	ryanodine receptors
SICI	short-interval intracortical inhibition
SMA	supplementary motor area
TBS	theta burst stimulation
tDCS	transcranial direct current stimulation
TES	transcranial electric stimulation
TMS	transcranial magnetic stimulation

Publications

The following publications have emerged from this thesis:

*Huang YZ, Rothwell JC (2004) The effect of short-duration bursts of high-frequency, low-intensity transcranial magnetic stimulation on the human motor cortex. Clin Neurophysiol 115:1069-1075.

*Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell J (2004) Theta burst stimulation of motor cortex. Neuron. (in press).

*Edwards MJ, Huang YZ, Mir P, Rothwell JC, Bhatia KP (2003)Does Reduced Motor Cortical Plasticity in Non-manifesting Carriers of the DYT1 Mutation Protect Against Development of Clinical Symptoms? (submitted)

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Rothwell JC, Huang YZ (2003) Systems-level studies of movement disorders in dystonia and Parkinson's disease. Curr Opin Neurobiol 13:691-695.

Edwards MJ, Huang YZ, Wood NW, Rothwell JC, Bhatia KP (2003) Different patterns of electrophysiological deficits in manifesting and non-manifesting carriers of the DYT1 gene mutation. Brain 126:2074-2080.

Rothwell JC, Edwards M, Huang YZ, Bhatia KP (2003) Physiological studies in carriers of the DYT1 gene mutation. Rev Neurol (Paris) 159:880-884.

Mir P, Huang YZ, Gilio F, Edwards MJ, Berardelli A, Rothwell JC, Bhatia KP (2004) Abnormal cortical and spinal inhibition in paroxysmal kinesigenic dyskinesia. Brain (in Press)

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* Incorporated into the thesis.

Chapter 1 Introduction

The nervous system has an extremely flexible structural and functional organisation. This flexibility allows the nervous system to transform itself and to analyse, store, and react to changes inside the body or to stimuli outside the body, properly and efficiently. This kind of complex adaptation seems to continue to occur through life from the embryo onwards although the mechanisms may differ at different times during development (Kemp et al., 2000; Bimonte et al., 2002; Crutcher, 2002). This process underlies our ability to adapt to and meet challenges in our life. An obvious example of the flexibility of the nervous system is learning and memory. It is astonishing how much people can learn and remember throughout their whole life. No machine in the world can match the many functions performed by humans effortlessly every day and every second of their lives.

Starting with studies of learning and memory, people have explored the mechanisms of how the nervous system modulates itself to meet requirements of a changing environment. However, the field is no longer restricted to learning and memory. Many pathways in the brain have the potential to be changed. Thousands of papers related to this topic have been published and the number is still growing up every year (Malenka, 2003). Many phenomena related to these processes, including cellular and molecular mechanisms have been elucidated is last 20 years. However, there are still many questions that cannot be answered due to the limitation of our knowledge and techniques.

Most work exploring flexibility of the nervous system has been performed in

animal preparations. Nevertheless, people are very keen to understand how similar processes occur in human beings, because it could be invaluable, if through understanding basic mechanisms we could develop techniques to modulate nervous function. This would not only give us a better understanding of the physiology of human nervous system, especially the brain, but also potentially allow us to treat neurological diseases or improve brain function. The development of a non-invasive technique for stimulating the human brain, transcranial magnetic stimulation (TMS), has brought this expectation closer to reality. By using TMS, people can investigate the effects produced by modulating brain function non-invasively and painlessly.

1.1 PLASTICITY

Plasticity (or neuroplasticity) is the term used to describe the phenomenon that nervous system is capable of adapting to both internal and external demands. The synapse is believed to play the most important role in plasticity, and most studies on adaptation in the CNS have focussed on synaptic plasticity. By enhancing or suppressing the efficiency of transmission between a presynaptic and a postsynaptic neuron, a certain degree of plasticity can be produced. The modulation can simply happen between a presynaptic terminal and a postsynaptic cell. This kind of plasticity is termed homosynaptic plasticity. When the modification of a synaptic strength is caused by a third neuron, it is called as heterosynaptic plasticity,

1.1.1 The synapse

Synapses are junctions between two neurones or between neurones and other

excitable cells where signals are usually transmitted from one cell to the other with high spatial precision and speed. Synapses can be either electrical or chemical, depending on whether transmission occurs via direct propagation of the electrical stimulus from the presynaptic process or via a chemical intermediate. In chemical synapses, the synaptic interface is formed by a close apposition of specialised regions of the cell membranes of the two participating cells. The presynaptic terminals contain a cluster of vesicles filled with neurotransmitters, whereas the postsynaptic plasma membranes are occupied with receptors that respond to neurotransmitters. The presynaptic part is commonly localised at the terminal branch of an axon and the postsynaptic part is at the surface of the cell body or of a dendrite. The space between the presynaptic and postsynaptic endings is called synaptic cleft, which is about 20nm wide. In the central nervous system (CNS), synapses can be classified into two groups: type I and type II or asymmetric and symmetric, respectively. There is evidence that at most sites type I synapses are mainly inhibitory synapses mediated by γ-aminobutyric acid (GABA), whereas type II are excitatory synapses mediated by glutamine. The structure of the synapse is designed to have maximal spatial precision and rapid speed of transmission (De Camilli et al., 2003). The presynaptic terminal releases only selective vesicles at active zones that are located in close proximity to clusters of postsynaptic receptors to improve the spatial precision. There are also mechanisms that prevent or reduce the lateral diffusion of neurotransmitters. The speed is achieved presynaptically by having a pool of vesicles ready to release coupled to a rapid stimulus-secretion mechanism and postsynaptically by fast chemoelectrical transmission mediated by ionotropic receptors. Synapses may also have a slow component of transmission that may be less spatially selective. Metabotropic receptors are sometimes localised more diffusely in the postsynaptic neuron and produce a slow synaptic response that can persist for seconds or minutes.

The neurotransmitters are synthesised in the region close to the cell body. In some neurones, the cell body is some distance from the synapse and transport between the two sites is a potential problem. The time required for macromolecules and organelles to travel to the periphery of the axon can be much longer than the rapid transmission of electrical signals. Simple diffusion can guarantee neither the delivery of neurotransmitters to the axon terminal, nor efficient retrograde flow for the recycling and degradation of materials. The axonal flow, anterograde or retrograde, is therefore assisted by a large number of molecular motors, which use microtubules or actin as trackers (Kuznetsov et al., 1992; De Camilli et al., 2003). Nevertheless, it may still take hours or days to reach the periphery of the axon. To cope with this problem, nerve terminal proteins have a prolonged half-life in the axon. Finally, the presynaptic terminal can recycle neurotransmitters to reform synaptic vesicles rapidly without involving the protein-synthesising machinery confined to the cell body. In addition, newly synthesised synaptic vesicle proteins are transported from the cell body to the nerve terminal in precursor membranes rather than as mature synaptic vesicles (Yonekawa et al., 1998; De Camilli et al., 2003).

The postsynaptic compartment includes a patch of plasma membrane containing a packed array of neurotransmitter receptors and the postsynaptic density (PSD), a structure which consists of an aggregation of neurotransmitter receptors and signalling proteins essential for chemical synaptic transmission. The receptors in this patch may be arranged heterogeneously. For instance, two types of receptors, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, are distributed in the central and peripheral regions at glutamatergic synapses, respectively (Kharazia and Weinberg, 1997). The

postsynaptic membrane also contains other proteins required for the formation and maintenance of the synaptic junction. The PSD is a specialization of the cytoskeleton at the synaptic junction. It lies adjacent to the cytoplasmic face of the postsynaptic membrane, in close apposition to the active zone of the synapse and the docked synaptic vesicles in the presynaptic terminal. Emerging evidence indicates that the PSD has multiple functions and is different in molecular composition and morphology at inhibitory and excitatory synapses.

1.1.2 Synaptic plasticity

As mentioned by Cajal in 1849, 'mental training cannot better the organisation of the brain by adding to the number of cells...' He proposed that the formation of new collaterals and protoplasmic expansions could reinforce pre-existing connections. Since Sherrington first introduced the term synapse in 1897, the idea that information could be stored in the brain by changing the strength of synapses has been gradually formulated. This kind of assumption was refined in Hebb's postulate (Hebb Donald, 1949), which has proved to be one of the most influential hypotheses in modern neuroscience. He proposed that the coordinated firing of pre- and postsynaptic cells could strengthen the synapse between them. Effectively this was a way of storing information and could directly explain phenomena such as learning and memory.

Habituation, a simple form of learning, was first described by Pavlov and Sherrington in 1927. An animal first responds to a new stimulus by attending to it with a series of orientating responses. If the stimulus is neither beneficial nor harmful, the animal learns to ignore it after repeated exposure. It was later demonstrated in cats' spinal cord that habituation leads to a decrease in the strength of the synaptic

connection between excitatory interneurones and motor neurones. This phenomenon and other forms of simple learning, e.g. sensitization and classical conditioning, were further studied systemically in the marine snail Aplysia, which has a simple nervous system containing only about 20,000 central nervous cells. Here it was found that the changes in synaptic strength can persist for one or more days and, more importantly, parallel the time course of the memory process (Kandel Eric, 1976). The experiments showed for example that a single session of 10 tactile stimuli leads to a short-term memory for habituation of the Aplysia gill withdrawal reflex lasting several minutes. Prolonged training without rest between training sessions produces a robust short-term memory, but the effect does not last much longer. Repeated short sessions of training separated by periods ranging from several hours to one day produce a long-term memory lasting as long as 3 weeks.

The short-term memory due to short-term plasticity and long-term memory caused by long-term plasticity are thought to have independent but overlapping processes that blend into one another. Both of them change the strength of connections at several synaptic sites and may share some mechanisms. For example, protein kinase A (PKA) and cAMP, intracellular second-messenger pathways that are critically involved in short-term plasticity, are also recruited for long-term plasticity. However, not all their mechanisms are shared and sometimes, short-term memory can behave differently to long-term memory. For example, in humans, epileptic seizure or head trauma affects long-term memory but not short-term memory, whereas Alzheimer's dementia starts with short-term memory deficit with preserved long-term memory. In experimental animal, inhibitors of protein or mRNA synthesis can selectively block long-term memory suggesting that new proteins may play an important role in stabilising memory over the long term.

1.1.3 Long-term potentiation

Unlike Hebb's postulate that associative stimuli are needed for changing the strength of synaptic transmission, Lømo, in 1966, reported that responses in dentate gyrus evoked by a single test shocks to the afferent perforant pathway were potentiated for a considerable time after short periods of repeated stimulation at 10-20 Hz (Bliss and Lomo, 1973). Bliss and Lømo in 1973 described the details for the first time and also termed it long-lasting potentiation, which is now commonly known as long-term potentiation (LTP). Since then, the publication of papers related to LTP has increased rapidly. One reason for this is the belief that LTP could be closely related to processes of memory and activity-dependent synaptic plasticity in mammalian brain. However, in spite of massive research on LTP, it has proved difficult to understand all the details that underlie its cellular and molecular mechanism. Most experiments have studied LTP at excitatory synapses in the CA1 region of hippocampus, although it has also been demonstrated in other kinds of synapses (see Lynch, 2004 for review). The transmitter at these excitatory synapses is glutamate. It has been demonstrated that LTP in excitatory synapses of the hippocampus is very similar (or even identical) to the LTP revealed at glutamatergic excitatory synapses throughout the mammalian brain.

Three basic properties of LTP were elucidated in the 1970s and early 1980s (Malenka, 2003). First, LTP induction is input-specific, which means that when LTP is produced at a single or one set of synapses, the increase in synaptic efficiency does not occur in adjacent synapses that were not activated by the protocol. Second, LTP induction is associative, which means that if LTP is induced at one set of synapses, it

can facilitate induction of LTP at a different set of synapses, even if the latter on its own was subthreshold for any effect. This effect occurs only within a limited temporal window at and just after the time at which LTP is induced. As noted below, this property is explained by the fact that strong activation at one site can cause depolarization at distant parts of the dendritic tree and hence influence development of LTP at local synapses. Third, LTP usually shows "co-opertivity", that is, a certain number of presynaptic inputs must be activated at the same time in order for it to occur. Later experiments have shown that processes in both pre- and post-synaptic terminals contribute to the change of the strength of synapses seen in LTP. Indeed, long-lasting LTP can also be accompanied by an increase in number of synapses.

1.1.4 Long-term depression

Long term depression (LTD) describes the functional opposite of LTP, a long lasting suppression in synaptic effectiveness that outlasts the period of induction. Such an effect was first observed by Lynch as a by product of LTP. He found that, in the CA1 region of the hippocampus in vitro, when LTP-inducing stimuli were delivered to one pathway resulted in a reversible depression in the nontetanised pathway. However it was not until 1992 that it was reported that homosynaptic LTD could be induced by low frequency stimulation (LFS) (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Since then, people have found that LFS can abolish LTP (Staubli and Lynch, 1990; Fujii et al., 1991), a phenomenon now known as depotentiation, which has slightly different mechanism from the mechanism of LTD in a naive synapse (i.e. a synapse without prior induction of LTP). Although LTD has been demonstrated at many synapses in the brain, the LTD at cerebellar parallel fiber-Perkinje cell synapse and at the hippocampal Schaffer collateral/commissural-CA1 pyramidal cell synapse

are the two best-understood forms.

If LTP is truely the basis of learning and memory in mammalian brain, what would be the role LTD plays in the function of brain? The physiological function of LTD has not been very clear so far. Many believe that LTD may work together with LTP to underlie learning and memory. For example, it could be by reducing the effectiveness of inhibitory synapses (at GABA synapses), or by "focusing" the effect of LTP to more specific subsets of synapses. It has also been proposed as a "neuronal substrate of forgetting" (Tsumoto, 1993). However, there is no strong support for this argument. On the other hand, there is some evidence showing that LTD may have some functional effects on behavioural learning in cerebellum (Shibuki et al., 1996; Lisberger, 1998) and also probably underlies the mechanism of spatial learning (Kemp and Bashir, 2001).

1.1.5 Repetitive stimulation and plasticity induction

There are very many ways of inducing plasticity naturally or artificially. Among them, repetitive electrical stimulation first introduced by Lømo and Bliss in late 60s to early 70s is the most efficient way of doing it, and is still the most commonly used protocol. However, other methods are now available, mainly due to our increased understanding of the basic mechanisms of synaptic plasticity. Thus, introduction of special molecules into the postsynaptic neuron or changes in its intracellular ion concentrations can now be employed to produce postsynaptic LTP or LTD. Chemically induced LTD uses phosphorylation-site-specific antibodies that can dephosphorylate Ser^{845} at Glu1 to induce LTD (Lee et al., 1998; Lee et al., 2000). Photolysis of Ca^{2+} involves photolysis of injected postsynaptic caged Ca^{2+}

compounds to change the level of $[Ca^{2+}]i$ to produce LTP or LTD (Neveu and Zucker, 1996; Yang et al., 1999).

LTP, in Bliss and Lømo's experiment (Bliss and Lomo, 1973), was produced by a short train of repetitive stimuli at a frequency of 10-20 Hz for 10-15 seconds or at 100 Hz for 3-4 seconds. On contrary, stimulation at a frequency between 1 and 5 Hz caused 'depotentiation' of LTP (Staubli and Lynch, 1990) or LTD in naïve synapses (Dudek and Bear, 1992). This led to the general idea that high frequency repetitive stimulation (HFS) can induce LTP, while low frequency stimulation (LFS) produces LTD. A perusal of the literature though shows that the precise pattern of stimulation used by different experimenters to induce LTP and LTD varies considerably both between labs and between the CNS systems under study.

1.1.5.1 Protocols for LTP induction

An early modification of the original protocol of Bliss (a long train of high frequency stimulation lasting for several seconds) was to give a few shorter trains intermittently. For example, four trains at a frequency of 50 or 100 Hz for 1-2 seconds applied every 10 seconds were given to induce LTP in rat prefrontal neurones (Hirsch and Crepel, 1990). However, this kind of stimulation with a regular frequency is less frequently used for LTP-induction nowadays. Most work uses patterned stimulation, now known as theta burst stimulation (TBS) to produce LTP. This evolved from observations of the natural firing pattern of pyramidal cells in the cat hippocampus that showed spontaneous activity consisting of an admixture of solitary spikes and bursts (Kandel and Spencer, 1961).Each burst contains around 4 spikes at 100-200

Hz and is repeated in the theta frequency range (4-7 Hz) (Eichenbaum et al., 1987). Based on this firing in theta rhythm, Larson and Lynch and colleagues (Larson and Lynch, 1986; Larson et al., 1986) developed the prototype of theta burst stimulation (TBS) to induce synaptic potentiation. The most widely used variety of TBS consists of a burst of four stimuli at 100 Hz, repeated 10 times at 5 Hz. The train is then repeated every 10 seconds for 10 cycles. It does not seem to be clear why investigators use 10 intermittent trains instead of a continuous long train to do TBS. The most likely reason is that Larson et al. (1986) found that the potentiation effect induced by a train of 20 bursts was much less than that induced by a train of 10 bursts. If this is the reason, the 10 repeated trains are actually 10 sessions of 10-burst stimulation. Although TBS is effective for producing LTP, other protocols of high frequency stimulation are in use and are effective for LTP induction, e.g. an 8-pulse train at 300 Hz given every 10 seconds for 30 times (Froc et al., 2000).

1.1.5.2 Protocols for LTD induction

Unlike protocols for LTP induction, which have changed a lot and are usually complex, protocols for LTD-induction are relatively simple and have not changed a great deal from the protocol first reported. Regular LFS at 1 Hz for 15 minutes (Kirkwood et al., 1993; Froc et al., 2000; Lee et al., 2000), at 2 Hz for 5 or 10 minutes (Hess and Donoghue, 1996; Urban et al., 2002), or at 5 Hz for 3 minutes (Zakharenko et al., 2002) are often used. Sometimes, a paired-pulse 1Hz protocol, which consists paired-pulses of 50 ms interstimulus interval repeated at 1 Hz for 15 minutes, has been used (Lee et al., 2003). LTD-induction protocols, which usually take several minutes to work, are actually very inefficient as compared with

LTP-induction protocols that only need seconds. The reason why people did not change the protocols is unclear. However, Heusler et al. (2000) has demonstrated that LTD could be induced by a 13-pulse train at 300 Hz given every second for 100-150 minutes. Although it still took several minutes to produce LTD, at least they have shown that it is not necessary to use LFS for LTD-induction.

1.1.5.3 Other protocols for inducing either LTP or LTD

The postsynaptic membrane potential is very important for the induction of plasticity (Artola et al., 1990). A train at 100 Hz lasting for 1 second repeated every 10 seconds for 3 times induced LTP when the postsynaptic membrane potential was at the level of resting membrane potential (-70 mV), whereas the same stimulation led to LTD when the membrane potential was hyperpolarized to -85 mV (Randic et al., 1993). A similar phenomenon also happens with LFS. A 2 Hz stimulation for 50 seconds induced LTP, LTD, or nothing when the postsynaptic membrane potential was > -20 mV, between -20 and -40 mV, or ≤ -40 mV, respectively (Ngezahayo et al., 2000).

Interestingly, although it is very difficult to find the reason why people started to use the various protocols to induce LTP and LTD many of them now can be explained by the theory of calcium influx, which is thought to be the 'key' to trigger plasticity. It is believed that high frequency stimulation elevates $[Ca^{2+}]i$ to a higher level or induces a quicker Ca^{2+} influx to induce LTP. In contrast, low frequency stimulation, which is usually longer in duration, causes prolonged and moderate increase of $[Ca^{2+}]i$ that preferentially produces LTD.

1.1.5.4 Plasticity induction in living animals

Most plasticity experiments were done in brain slices from dead animals which are disconnected from other parts of the brain. In order to test whether similar mechanisms occur in the intact state, people started to try to induce plasticity in anesthetised animals. Luckily it turns out that similar effects can be observed. Repetitive short trains of HFS at up to 400 Hz or TBS induce LTP in the hippocampus of anesthetised animals (Charpier and Deniau, 1997; Heynen and Bear, 2001; Abe et al., 2003; Fukazawa et al., 2003; Izaki et al., 2004). There is also no problem to induced LTP in the hippocampus of free moving rats with a protocol containing a 15-pulse train at 200 Hz given every 200 ms for 10 times and then repeated every 10 seconds for 10 cycles (Manahan-Vaughan et al., 2003). On contrary, a train of 900 pulses at 1 Hz (Izaki et al., 2004) or a 5-pulse burst at 250 Hz given every second for 180-190 seconds (Takita et al., 1999) were used to induce LTD. It has been suggested that the direction of plasticity in the hippocampus may be regulated by the phase of tail pinch-triggered theta rhythm (Holscher et al., 1997). Five pulses at 200 Hz given on the positive phase of the theta rhythm reliably induced LTP, whereas bursts given on the negative phase depotentiated the LTP, although no LTD was produced.

It is known to be more difficult to induce LTP in the neocortex than in the hippocampus even in brain slice preparations (Hess et al., 1996). GABAergic blockers and/or young animals are usually needed in addition for LTP to be clear. It is even more difficult to induce LTP in the neocortex of free moving animals. It requires spaced and repeated sessions applied over a period of days to induce LTP (Trepel and Racine, 1998). To induce LTD in the neocortex of free moving animals

using a train of 900 pulses at 1 Hz also requires multiple stimulation sessions to reach a reliable level (Froc et al., 2000).

1.1.6 Mechanisms of synaptic plasticity

The ability of synapses to undergo these short-term or long-term changes of synaptic efficiency has been demonstrated throughout the mammalian brain, including hippocampus, cerebellum, striatum, neocortex, thalamocortical projections... and many other areas or pathways (Calabresi et al., 1992; Hess and Donoghue, 1996; Herry and Garcia, 2002; Schutter et al., 2003). Current understandings of the mechanism of plasticity are mainly based on glutamatergic excitatory synapses, although there are some reports showing that similar phenomena occur at GABAergic inhibitory synapses (see Gaiarsa, 2004 for review). Pre- or postsynaptic terminals or both may contribute the change of the strength of synaptic efficiency, although, generally speaking, short-term plasticity favours presynaptic mechanisms, while long-term plasticity may recruit both pre and postsynaptic mechanisms.

1.1.6.1 Presynaptic mechanisms of plasticity (I) *very short lasting effects*

Very short lasting presynaptic plasticity has been noted for more than 50 years. Activity of the presynaptic nerve can produce short-term changes in synaptic efficiency lasting milliseconds to several minutes. Presynaptic plasticity is usually due to a change in the amount of transmitter released by each presynaptic action potential. Analysis of the changes in statistics of transmitter release demonstrated that short-term potentiation reflects an increase in the probability of release of

available quanta, with perhaps also an increase in the number of release sites capable of releasing a quantum (Zucker and Regehr, 2002). On contrary, most models of short-term depression are based on the idea that it reflects depletion of a pool of vesicles that are poised and ready for release.

Changes in probability of transmitter release are thought to be caused by changes in the concentration of intracellular calcium ($[Ca^{2+}]i$) due to depolarisation of the presynaptic terminal by invasion of action potentials as well as by activation of presynaptic Ca^{2+} channels (De Camilli et al., 2003). Diminished Ca^{2+} influx in many forms of presynaptic inhibition has been demonstrated by Ca^{2+} imaging techniques (Wu and Saggau, 1997), whereas increased presynaptic calcium levels are associated with presynaptic facilitation. Presynaptic inhibition can also induced by applying a substance such as FMRFamide (Phe-Met-Arg-Phe-NH_2) to inhibit a site downstream of Ca^{2+} influx (De Camilli et al., 2003). This supports the hypothesis that regulating steps in synaptic vesicle cycling can also induce presynaptic plasticity.

The presynaptic Ca^{2+} influx may activate the calcium/calmodulin-dependent protein kinases, CaMKI and CaMKII, and maybe also cAMP-dependent protein kinase (Malenka and Siegelbaum, 2003). These protein kinases can phosphorylate synapsin, which may anchor synaptic vesicles to the cytoskeleton in its dephosphorylated form (Greengard et al., 1991), to enhance vesicle mobility and cause short-term potentiation. The molecular mechanism of short-term depression is less well understood. It could be due to presynaptic vesicle depletion (Geppert et al., 1997). Depletion of synapsin II alone or of synapsin I and II together, which may reduce the total pool of synaptic vesicles, also leads to a marked increase in the rate of development and extent short-term depression (Rosahl et al., 1995).

1.1.6.2 Presynaptic mechanisms of plasticity (II) *longer term effects*

LTP between hippocampal mossy fiber synapses and dentate granule and CA3 pyramidal cells, is expressed mainly via presynaptic mechanisms (Reid et al., 2004), although postsynaptic LTD was also reported in this pathway (Lei and McBain, 2004). It can be demonstrated using the fluorescent dye FM 1-43 which is taken up into synaptic vesicles in presynaptic terminals in an activity dependent manner as a result of endocytosis. Subsequent presynaptic stimulation leads to the release of stained vesicles by exocytosis. The rate of the release of FM 1-43 thus provides a direct index of presynaptic function. Using FM 1-43, it has been demonstrated that presynaptic function was enhanced in LTP (Zakharenko et al., 2001) but suppressed in LTD (Zakharenko et al., 2002). People believe this is most likely due to changes in the size of quantal transmission which can be regulated at many possible sites, including alterations to fusion pore kinetics (Zakharenko et al., 2002; Choi et al., 2003; Liu, 2003; Stanton et al., 2003)

1.1.6.3 Postsynaptic mechanisms of plasticity (I) *very short lasting effects*

One type of rapid postsynaptic plasticity is desensitisation (Jones and Westbrook, 1996). Exposure of ligand-gated channels to an agonist can lead to channel opening and can also put some of the channels into a nonresponsive state. This process is called desensitization. It can take tens of milliseconds or even minutes for channels to recover from such a desensitised state. A second postsynaptic mechanism can also contribute to very short-term plasticity. Synaptic connections between pyramidal

neurones and postsynaptic multipolar interneurones in layer II/III of rat neocortex are mediated by AMPA receptors. These receptors are blocked by endogenous intracellular polyamines that are present in almost all cells. In excised patches, this polyamine block is relieved by depolarisation. This relief of polyamine block thus enhances the responses of AMPA receptors and can lead to a postsynaptic form of facilitation (Rozov et al., 1998; Rozov and Burnashev, 1999).

1.1.6.4 Postsynaptic mechanisms of plasticity (II) *longer term effects*

It is widely, although not universally, believed that a postsynaptic locus for the expression of plasticity is more important for the modulation of synaptic transmission and also for maintaining long-term plasticity than presynaptic sites. Most studies exploring the mechanism of plasticity, therefore, focus on postsynaptic mechanisms. The underlying mechanisms of LTP/LTD are apparently complicated. In addition there are so many methods to induce plasticity and it is not known whether all of them have the same or partially different mechanisms. The mechanisms we understand most are based on activity-dependent plasticity, which can be induced by repetitive stimulation. More than a hundred molecules have been implicated in LTP/LTD ((Sanes and Lichtman, 1999). Not all of them, of course, are directly linked to the expression of synaptic plasticity; some of them control the expression of others. The most commonly accepted mechanism of LTP/LTD in excitatory pathway involves glutamate receptors, calcium, and AMPA receptor regulation involving some protein kinases.

Glutamate receptors

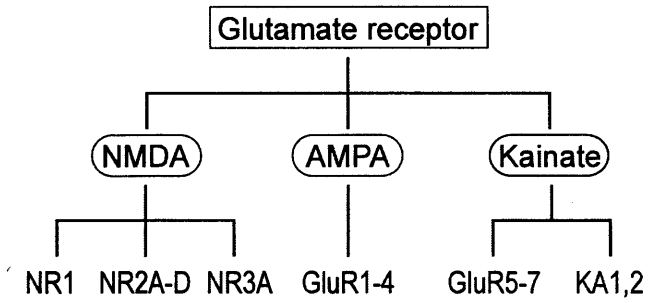

Fig 1.1 ***Classification of iontropic glutamate receptors***

Glutamate receptors are examples of the two major superfamilies of receptors, the ligand-gated ion channel receptors (LICR) and the G-protein-coupled receptors (GPCR). These groups are usually referred to as ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). There are mainly three groups of ionotropic glutamate receptors, i.e. α-amino-3-hydroxy-5-methyl-4-iso-xazolepropionic acid (AMPA) receptors, *N*-methyl-D-asparatate (NMDA) receptors and kainate receptors, located in the postsynaptic membrane. Each of them interacts with the glutamate released from the presynaptic terminal in different ways (Sheng and Kim, 2002). Each type of receptor contains several subunits (Fig 1.1). All ionotropic glutamate receptor subunits share a common basic structure. Like other ligand gated ion channels, such as the $GABA_A$ receptor, the ionotropic glutamate receptor subunits possess four hydrophobic regions within the central portion of the sequence. Kainate receptors constitute a separate group from the NMDA and AMPA receptors, although they share many of the same structural characteristics. They have classically been implicated in epileptogenesis. They have also been demonstrated to be important for plasticity induction in some cerebral regions, e.g. the mossy fibre synapse in the CA3 region of the hippocampus. The metabotropic glutamate (mGlu)

receptors, which respond more slowly to glutamate, are G-protein coupled receptors (GPCRs) that have been subdivided into three groups, Group I, II and III, based on sequence similarity, pharmacology and intracellular signalling mechanisms.

When activated by glutamate, the AMPA receptor opens an ion channel in the membrane that is permeable to sodium and potassium. Influx of sodium then depolarises the postsynaptic membrane. At resting potential the ion channel of the NMDA receptor is blocked by Mg^{2+}, but when the membrane is depolarised the Mg^{2+} dissociates from the receptor and the channel allows influx of Ca^{2+} and Na^{+} into the cell. The subtypes of NMDA receptors may govern the direction of synaptic plasticity in hippocampus (Liu et al., 2004). Selectively blocking the subtype containing NR2A subunit abolished the induction of LTD but not LTP, while the preferential inhibition of NR2A-containing subtype prevents the induction of LTP without affecting LTD induction. Activation of the metabotropic glutamate (mGlu) receptor leads to production of inositol triphosphate ($InsP_3$), which will trigger $InsP_3$ receptors to release Ca^{2+} from internal stores.

When a presynaptic terminal is activated during an LTD induction paradigm, glutamate is released and AMPA receptors are activated to increase the permeability for Na^{+} influx and K^{+} efflux to induce rapid excitatory postsynaptic currents (EPSC). When the postsynaptic membrane is depolarised, extracellular Mg^{2+}, which blocks the NMDA-dependent channels when the membrane is not depolarised, is dissociated from the voltage dependent NMDA receptor to allow Ca^{2+} and also Na^{+} to enter the dendritic spine. The mGlu receptor, which responds more slowly, is also triggered to release $InsP_3$ to induce Ca^{2+} release from internal stores, which in turn causes further Ca^{2+} release through ryanodine receptors (RyRs).

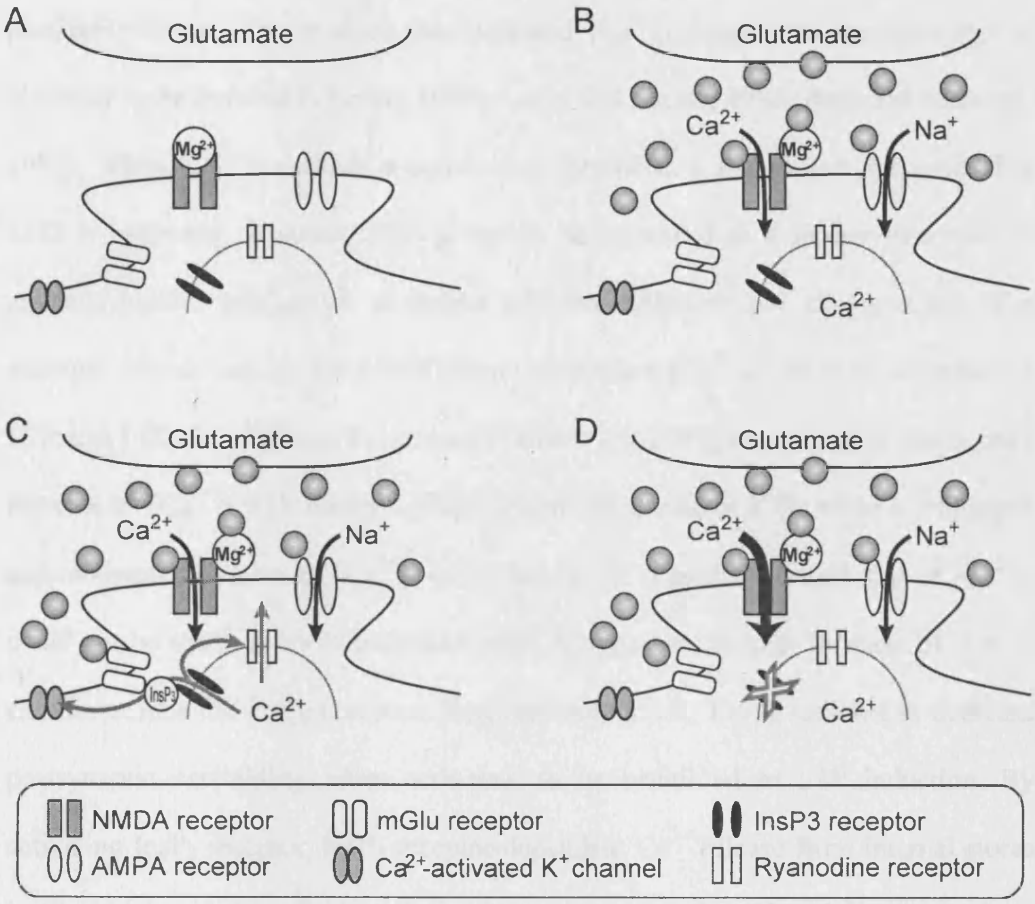

Fig 1.2 ***A model for the postsynaptic Ca^{2+} influx at a glutamatergic synapse.*** *A, An inactive glutamatergic synapse. B, The presynaptic terminal is activated to release vesicles of glutamate to trigger AMPA receptors to open Na^{+}/K^{+} channels causing depolarisation of the postsynaptic membrane potential, which activates the NMDA receptors to allow Ca^{2+} influx. C, mGlu receptors are also activated by glutamates to release InsP3 to release Ca^{2+} from endoplasmic reticulum. The release of Ca^{2+} from internal stores makes more Ca^{2+} being released through ryanodine receptors. The further release of Ca^{2+} can reduce postsynaptic neuronal excitability through Ca^{2+}-activated K^{+} channels. D, A high-level of Ca^{2+} transient increase desensitises $InsP_3$ receptors.*

Calcium

Calcium is believed to be an important key that determines the direction of change in synaptic efficiency. In many systems, an elevation in the level of postsynaptic intracellular calcium concentration ($[Ca^{2+}]i$) triggers both LTP and LTD. One

possibility is that the level of the increased $[Ca^{2+}]i$ determines the direction of plasticity to be induced (Lisman, 1989; Artola and Singer, 1993; Bear and Malenka, 1994). When $[Ca^{2+}]i$ exceeds a certain low threshold, a mechanism for producing LTD is activated. Whereas, $[Ca^{2+}]i$ has to be increased to a higher threshold to activate another mechanism to induce LTP. Nevertheless, not all agree this. For example, Neveu and Zucker (1996) found no distinct $[Ca^{2+}]i$ threshold for inducing LTP and LTD. In addition, Yang et al. (Yang et al., 1999) demonstrated that a brief increase of $[Ca^{2+}]i$ with relatively high magnitude produces LTP, while a prolonged and moderate elevation of $[Ca^{2+}]i$ can induce LTD. A prolonged high rise of $[Ca^{2+}]i$ could not be studied due to technical issue. A transient but high increase of $[Ca^{2+}]i$ can desensitise the $InsP_3$ receptor (Bezprozvanny et al., 1991), which can decrease postsynaptic excitability when activated, to be beneficial to LTP induction. By activating $InsP_3$ receptor, $InsP_3$ receptor-dependent Ca^{2+} release from internal stores is provoked to induce further Ca^{2+} release through RyRs. The Ca^{2+} release from internal stores may reduce the activity of postsynaptic cells through Ca^{2+}-activated K^+ channels (Cordoba-Rodriguez et al., 1999). Moreover, it is believe that activated $InsP_3$ receptors can decrease the activity of NMDA receptors through unknown mechanisms (Nishiyama et al., 2000). Therefore, the spatiotemporal pattern of calcium increase seems to be crucial in affecting the direction of synaptic plasticity.

Regulation of AMPA receptor

Although the plasticity effect is initiated by an increase of $[Ca^{2+}]i$, a series of downstream consequences produces and maintains the effect. Among them, the regulation of the activity of AMPA receptors in the postsynaptic membrane is an important and well-understood process. AMPA receptors contain four subunits, Glu1 to Glu4. Phosphorylation of Glu1 occurs in LTP induction to increase the single

channel conductance of AMPA receptors (Barria et al., 1997; Lee et al., 2003), whereas dephosphorylation of Glu1 happens in LTD induction to decrease the activity of AMPA receptors (Kameyama et al., 1998; Lee et al., 2003). The site where phosphorylation/dephosphorylation happens is different (Lee et al., 2000), although they both occur in Glu1. In naïve synapses, LTP leads to an increase in phosphorylation at the calcium-CaM-dependent protein kinase II (CaMKII) site (Ser^{831}, also a PKC site), and LTD leads to dephosphorylation of the protein kinase A (PKA) site (Ser^{845}).

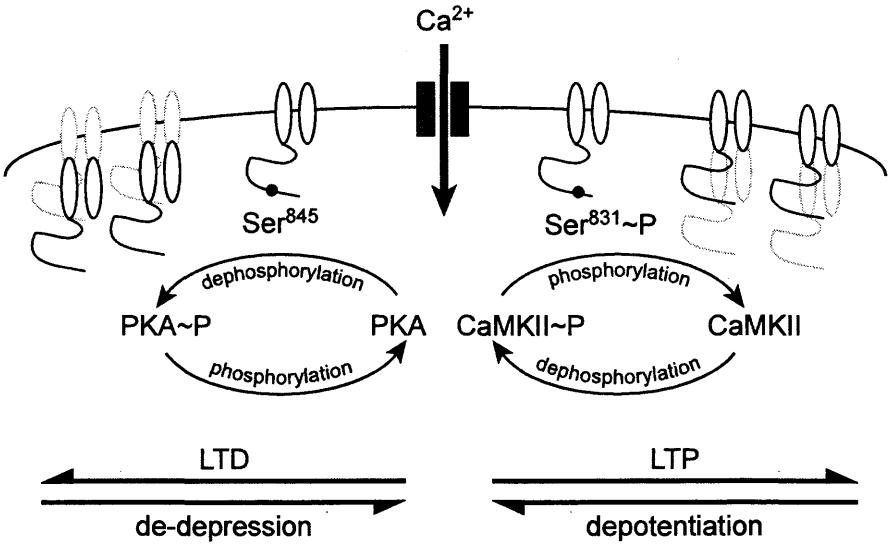

Fig 1.3A ***simplified diagram of AMPA receptor regulation.*** *Ca^{2+} influx activates protein kinases (e.g. CaMKII, PKC and PKA) to phosphorylate/dephosphorylate Glu1 subunit of AMPA receptors and changed the excitability of the receptors. The site phosphorylated depends on the history of the synapse. The exocytosis and endocytosis of AMPA receptors may also contribute the change of the postsynaptic excitability.*

Exocytosis of AMPA receptors during LTP (Sheng and Lee, 2001) and the endocytosis of AMPA receptors during LTD (Carroll et al., 2001) has also been reported. The exocytosis of Glu1-containing AMPA receptors, resulting in synaptic potentiation, is induced by the activation of NMDA receptors and CaMKII (Hayashi

et al., 2000). The AMPA receptor endocytosis is clathrin-dependent and is mediated in part by secondary activation of voltage-dependent calcium channels, and in part by ligand binding independent of receptor activation (Lin et al., 2000; Man et al., 2000). By modifying the activity and the number of AMPA receptors in the postsynaptic membrane, the strength of synapses can be changed, because AMPA receptors mediate the EPSC in glutamatergic synapses.

The structural changes

The responses to the induction of plasticity at the molecular level are accompanied by dramatic structural and anatomical changes. These involve the redistribution of protein or even newly synthesised protein in pre- and postsynaptic terminals (Antonova et al., 2001). In addition, visible changes in the morphology of dendritic spines (Leuner et al., 2003) and axon terminals, profiles of active zones and postsynaptic densities and even the number of synapses, can happen as early as 10 minutes after stimulation (Engert and Bonhoeffer, 1999; Toni et al., 1999; Geinisman, 2000). Reorganisation of actin could contribute to the structural change of spines that accompanies plasticity (Lamprecht and LeDoux, 2004). This is supported by the fact that administration of a drug that blocks actin polymerisation can reduce LTP. AMPA receptors also contribute to stabilise the structural change (Lamprecht and LeDoux, 2004). Outside the synapse, interactions between cells and the extracellular matrix (ECM) have also been accepted to be crucial in neural development and reorganisation (Dityatev and Schachner, 2003). This may be because ECM molecules couple to the cytoskeleton and tyrosine kinase activities through receptors on the cell surface and also interactions with ion channels or neurotransmitter receptors.

1.1.7 Associative plasticity- Hebb's postulate

The synaptic modulation that was originally postulated by Hebb required correlated activity rather then just repetitive activity in a single pathway as discussed above for the protocols of LTP and LTD. Recent experiments have confirmed that correlated spiking of pre- and postsynaptic neurones can enhance or suppress synaptic connections, depending on the temporal order of spiking. This form of plasticity is also called associative plasticity. The temporal order of synaptic events is very important. When postsynaptic spikes are triggered 10 ms after the onset of subthreshold excitatory postsynaptic potentials (EPSPs) their efficiency can be enhanced, whereas when the spikes come earlier than EPSPs, LTD is induced (Markram et al., 1997). The mechanism of associative plasticity is not very clear. Magee and Johnston (1997) found the spikes that occur immediately after EPSPs can produce a significantly higher Ca^{2+} influx at the synaptic site, and it was suggested that this Ca^{2+} influx could result in LTP. These effects may well be related to the examples quoted above in which low-frequency presynaptic stimulation coupled with concurrent postsynaptic depolarisation can induce LTP in the hippocampus and many other cortical areas (Malenka and Nicoll, 1999).

The critical time window is relatively similar in many induction protocols in different pathways. During associative repetitive stimulation at 1 Hz (Bi and Poo, 2001), postsynaptic spiking within 20 ms after EPSP induces LTP, whereas that within about 20 ms before EPSP causes LTD. At Schaffer collateral synapses in the CA1 of rat hippocampus, associative stimulation with postsynaptic spiking within 15-20 ms after or before EPSPs produces LTP or LTD, respectively (Nishiyama et al., 2000). Hess et al. (Hess et al., 1996) also demonstrated that LTP in layer II/III

horizontal connection of the rat motor cortex, which usually needs focal application of bicuculline methiodide (Bic) to inhibit GABA mechanisms, could be induced without Bic by cotetanization of vertical pathways simultaneously with horizontal activation.

1.1.8 Is plasticity a real learning mechanism?

As pointed out above, much of the interest in synaptic plasticity is related to the fact that these mechanisms may underlie learning and memory. However, most work on plasticity is based on brain slices, which makes it difficult to evaluate its real physiological consequences. However, the classical properties of LTP/LTD may be relevant to associative or relational features of learning and memory. For example, the input-specificity allows greater storage capacity than would changes in cell excitability. The long-lasting effect can obviously be useful for long term memory. On the other hand, the theta burst stimulation, which mimics the natural firing pattern found in the hippocampus of rats or cats, can produce LTP in at least the CA1 region of the hippocampus (Larson and Lynch, 1986, 1989), suggesting that naturalistic patterns of activity can induce synaptic plasticity.

Rioult-Pedotti et al. (2000) demonstrated that synapses in the trained MI were near the ceiling of their modification range, compared with the untrained MI, but the range of synaptic modification was not affected by learning. In the trained MI, LTP was markedly reduced and LTD was enhanced. These results can be explained by the hypothesis that the strength of the synapses was enhanced by learning to the saturated range and therefore occluded further LTP induction. In the mean time, this enhanced synaptic strength also increases the range for depression. Alternatively, it is possible

to explain the findings by the Bienenstock–Cooper–Munro (BCM) theory of bidirectional synaptic plasticity (Bienenstock et al., 1982; Artola et al., 1990). If LTP were involved in a learning process, then threshold for the subsequent induction of LTP was increased by preceding learning and, concomitantly, threshold for LTD induction was decreased. These results provide evidence that the increased strength of synapses during learning is produced through LTP mechanisms. Similar results were also found in human subjects evaluated with the technique of transcranial magnetic stimulation (TMS) and the principles of the BCM theory is preferred to explain the mechanism (Ziemann et al., 2004).

1.2 PROBING PLASTICITY IN HUMAN SUBJECTS

1.2.1 Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive technique that can be used to stimulate central and peripheral nervous system. Michael Faraday, in 1831, demonstrated that a changing or 'time-varying' magnetic field, but not a static field, is capable of inducing electrical currents around it. The current induced by a magnetic field can, therefore, evoke nerve or muscle action potentials. Although the first experiment of a physiological effect caused by a time-varying magnetic field was demonstrated by d'Arsonval in 1896 (Barker, 2002), magnetic stimulation based on the technique we are currently using was not reported until 1985 (Barker et al., 1985). Before the technique of magnetic stimulation matured, people had experimented with electric stimulation through the scalp to stimulate the human brain. The first clinical application method of transcranial electric stimulation (TES) was

developed in 1980 (Rothwell, 1997). However, TES produces local discomfort and large muscle contraction on the scalp due to the electric current flows in the scalp. Only a small proportion of the current penetrate into the brain. Because TMS is much more comfortable, TES is used very little at the present time.

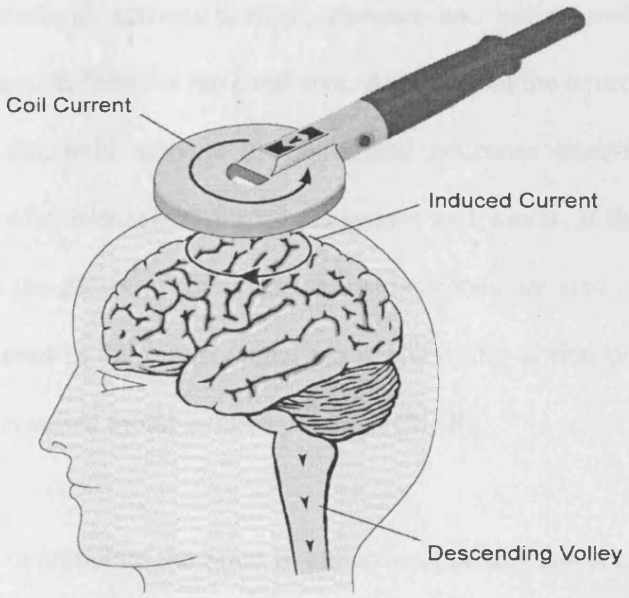

Fig 1.4 ***TMS coil and current induction****. A TMS coil can produce electrical current that has opposite direction to the current in the coil in the brain through an intact scalp to stimulate the brain.*

The magnetic stimulator contains a large electrical capacitance, which can be discharged in a very short time, to produce a large current flowing into the coil connected to it. Usually the current reaches a peak in 200 μs and then decays to zero more slowly. The current produces a time-varying magnetic field oriented perpendicular to the coil. The magnetic field produced can be as high as 3 Tesla (Barker et al., 1985), depending on the stimulator and the coil. The changing magnetic field, furthermore, induces current surrounding the field in any conductive objects nearby. The magnetic field is able to penetrate the skull to reach brain due to

the low magnetic impedance of the skull. Therefore, it can be used to stimulate the brain with little difficulty. The current induced in the scalp is much smaller compared to the current produced by TES, explaining why TMS turns out to be a much more comfortable technique to stimulate the brain. By applying a pulse of TMS over the motor cortex, the current produced by the quickly changing magnetic field can, when over a certain threshold, activate cortical neurones and initiate action potentials in corticospinal fibres. At least for the hand area, it seems that the neurones recruited by TMS at lowest threshold activate the pyramidal neurones trans-synaptically and generate a series of corticospinal discharges known as I-waves. If the TMS intensity is increased then the axons of the corticospinal neurones are also activated directly and lead to D-waves in the corticospinal tract. The motor action potential recorded from the muscle is called motor evoked potential (MEP).

The ability to stimulate the brain in conscious humans has lead to the idea that it might be possible to reproduce some of the experiments performed on synaptic plasticity in animals. However, it is first necessary to know how to monitor the effectiveness of cortical synapses in the human brain. The commonly used methods are listed below:

1.2.2 Measuring motor cortical excitability

As noted above, single pulse of TMS over primary motor cortex can activate a direct response (D wave) followed by a number of periodic indirect responses (I waves). The D wave could be produced by stimulating the initial segment of the axon or the proximal axon of cortical pyramidal cells. In the hand area, the lack of effect of contraction on the D wave indicates that the point excited to produce D wave could

be several nodes distant to the initial segment, perhaps at the point where the axons bend into the white matter before entering the internal capsule (Di Lazzaro et al., 1999; Rothwell, 2003). The I waves are produced by activating excitatory inputs synaptic to the pyramidal cells. The reason for the periodicity of the I wave input is not fully understood. It may depend to some extent on reverberating activity in synaptic circuits in the cortex, or it may depend upon the membrane properties of the pyramidal cell which cause it to fire repetitively after a large synchronous depolarising input. The I waves disappear when the grey matter of the brain is removed (Patton and Amassian, 1954), suggesting the source of these responses is within the cortex. One possible source is the network of cortical interneurones that synapse on pyramidal neurones. Another source might be the axon collaterals of other pyramidal neurones that run in layers III and V of the cortex. While using the commonly use figure-of-eight coil, the lowest threshold occurs when the coil is placed tangentially to the scalp with the handle pointing backwards and approximately perpendicular to the central sulcus (Mills et al., 1992). Such stimulation, which induces current in a posterior-anterior (PA) direction, provokes preferentially I1 wave (the first I wave). As the stimulus intensity increases, the I1 becomes bigger and then is followed by later I waves. The D wave can only be seen when the intensity is increased to 180-200% AMT (Di Lazzaro et al., 1998). Therefore, the amplitude of MEPs which is mainly dependent, especially at rest, on I wave input to motoneurones is generally used in many experiments as a parameter of motor cortical excitability. It is easier to recruit D waves with the coil orientation that induces current in the brain in a lateral to medial (LM) direction (Di Lazzaro et al., 1998). Anterior-posterior (AP) stimulation seems to be a complex process that may differ between subject (Di Lazzaro et al., 2001), although it has been suggested to recruit I3 wave earlier than I1 wave (Sakai et al., 1997).

Motor threshold is reduced and the amplitude of MEPs is increased when subjects contract the target muscle voluntarily during the tests. It has been suggested that the facilitation effect is mainly produced by an increased excitability at the spinal cord (Day et al., 1987), especially, when the contraction level is high. However, Mazzocchio et al. (1994) suggested that the effect could be also, at least partially, be due to an increase in descending volleys from a cortical level. Di Lazzaro et al. (1998) also demonstrated that voluntary contraction enhances I wave volleys in recordings of descending spinal cord volleys, while D wave volleys are not effected. These results suggest that the facilitation of MEPs by voluntary contraction is contributed to by both intraspinal and intracortical mechanisms.

1.2.3 Testing intracortical inhibitory and facilitatory circuits

There are now a large number of ways of examining intracortical circuits in human motor cortex using TMS. Those most commonly used will be described below:

1.2.3.1 Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF)

The paired-pulse TMS paradigm developed by Kujirai et al. (1993) is commonly used to study intracortical inhibitory and facilitatory circuits. When two pulses of TMS are given very close together, the first (conditioning) pulse delivered at a subthreshold intensity will modulate the size of MEPs evoked by a suprathreshold second (test) pulse (test-MEP). Short-interval intracortical inhibition (SICI) can be activated when the interstimulus interval (ISI) is around 1-5 ms, whereas intracortical

facilitation (ICF) happens when the ISI is at 8-20 ms. It is thought that SICI and ICF occur because of interactions at the level of the cerebral cortex (Kujirai et al., 1993). Thus, a subthreshold conditioning pulse has no effect on spinal H-reflexes at ISIs when the MEP is suppressed. Additional evidence comes from the observation that the responses evoked by TES (usually D-wave responses) are not inhibited by the conditioning pulse. Finally, cervical epidural recordings show that the conditioning pulse inhibits all I waves, except I1 at ISI of 1-4 ms (Di Lazzaro et al., 1998). The fact that the I1 is not suppressed indicates that the inhibition is not only cortical but also that it occurs before the synapse to the pyramidal cell.

Several experiments have suggested that the mechanisms for producing SICI and ICF are different. Firstly, as mentioned above, the threshold for producing SICI is slightly lower than the intensity for ICF. Secondly, the orientation of the coil for the conditioning stimuli does not affect the amount of SICI, but is critical for ICF. The facilitatory effect is prominent when the current is directed from posterior to anterior. Thirdly, CNS-active drugs can modulate SICI and ICF independently (Ziemann, 2002). Moreover, an intra-operative recording demonstrated that MEP evoked by subdural electrical stimulation could be inhibited or facilitated by a subthreshold conditioning delivered through neighbouring pairs of electrodes. The sites producing inhibition and facilitation were different. (Ashby et al., 1999) Finally, higher test intensities and test MEP amplitudes lead to a small increase in SICI, whereas ICF is not consistently affected by changes in test intensities (Chen et al., 1998).

It is suggested that SICI is produced by GABAergic inhibition (Chen et al., 1998; Hanajima et al., 1998; Ziemann et al., 1998). Moreover, SICI is enhanced by

Topiramate, a $GABA_A$-receptor agonist and non-NMDA-glutamate receptor antagonist, (Reis et al., 2002) but not affected by ion channel blockers (Ziemann et al., 1996) or $GABA_B$-receptor activators (Reis et al., 2002), suggesting SICI is mainly mediated by $GABA_A$ergic inhibition. However, it could actually be a complex phenomenon involving many different circuits (Fisher et al., 2002; Roshan et al., 2003). The mechanism of ICF, which may overlap with SICI (Hanajima et al., 1998), is still unclear.

A number of factors may affect intracortical inhibitory or facilitatory circuits. SICI and ICF were both decreased by voluntary contraction of the target muscle, but not by contraction of an ipsilateral proximal muscle (Ridding et al., 1995). The exact mechanism of the enhancement on MEPs (see above) and the decrease in SICI and ICF by voluntary contraction is still not clear. The decrease in SICI happens not only when test MEPs are evoked by PA stimulation (contributed by I_1 and later I waves), but also on MEPs evoked by AP stimulation (contributed mainly by later I waves) (Zoghi et al., 2003), suggesting that the decrease in SICI may be due mainly to effects on later I waves.

1.2.3.2 Long-interval intracortical inhibition (LICI)

Long-interval intracortical inhibition (LICI) can only be elicited by a suprathreshold conditioning pulse and happens when ISI is between 50 to 200 ms (Valls-Sole et al., 1992; Wassermann et al., 1996). It is very unlikely that the inhibition at ISI of this range is due to refractoriness of any neural element along the motor pathway. The refractory period of a motor neuron is probably less than 20 ms (Boniface et al., 1991). In addition, several lines of evidence suggest that LICI also relies, at least to

some extent, on events at the cortical level. First of all, although a suprathreshold stimulation could produce inhibition on H-reflexes or F-waves (Cantello et al., 1992; Triggs et al., 1993; Uncini et al., 1993; Ziemann et al., 1993), this lasted only for 50-100ms, after which spinal excitability was fully recovered. Second, although responses to TMS are suppressed 100-200ms after the conditioning pulse, responses to TES are not (Inghilleri et al., 1993). Furthermore, the I wave volleys during spinal recordings were suppressed at this interval by a suprathreshold TMS conditioning pulse (Nakamura et al., 1997), indicating the paired pulse inhibition is caused by intracortical inhibitory mechanism.

LICI may be due to $GABA_B$ inhibition, because Tiagabine, which inhibits the uptake of GABA from the synaptic cleft, enhances LICI (Werhahn et al., 1999; Sanger et al., 2001).

1.2.3.3 Short-interval intracortical facilitation (SICF)

Other than SICI, ICF, LICI mentioned above, another phenomenon called short-interval intracortical facilitation (SICF) or intracortical I-wave facilitation can be used for evaluating plasticity effects in humans. To obtain SICF, two pulses are given with a very small ISI (e.g. at an ISI of 1.5 ms). At such an interval, a suprathreshold conditioning pulse can facilitate the response to a subthreshold test pulse. This is believed to involve a mainly non-synaptic facilitation through direct excitation of the axon initial segment of interneurones along the late I-wave pathway by the second pulse which are made hyperexcitable through EPSPs produced by the first pulse (Ilic et al., 2002)

1.2.3.4 Silent period

The cortical silent period can be also used to test intracortical inhibitory circuits. When TMS is applied over the motor cortex while the subjects are maintaining voluntary muscle contraction of the target muscle, a pause in the ongoing EMG activity appears after the evoked MEP. This pause is called the cortical silent period. The duration is linearly related to the stimulus intensity, whereas the level of the voluntary contraction only causes minor changes in the duration. The mechanism of the silent period is complex and involves both segmental and cortical effects. The silent period due to a muscle twitch induced by peripheral stimulation lasts much shorter than the cortical silent period, indicating that spinal mechanisms can not explain the whole silent period (Cantello et al., 1992). The H-reflex was suppressed during the early phase of the silent period and recovered toward the end of the silent period (Fuhr et al., 1991). In addition, TMS could not evoke MEP and only smaller MEP could be provoked by TES in the first 50 ms of the silent period. Therefore, the early phase of the silent period is more likely caused by a spinal mechanism, while the later part is due to cortical suppression. Although the silent period can be reduced by diazepam, which is a $GABA_A$ receptor antagonist (Inghilleri et al., 1993), most evidence is in favour of a mechanism mediated by $GABA_B$ receptors (Siebner et al., 1998; Werhahn et al., 1999; Sanger et al., 2001).

1.2.4 TMS mapping

A different way of using TMS to probe the effects of plastic reorganization of the brain is the technique of motor cortex "mapping" (Pascual-Leone et al., 1999; Karl et

al., 2001; Schwenkreis et al., 2001).The motor cortex in primates is somatotopically organized. Although single pulse TMS can not provide the detail of somatotopy as seen after direct electrical stimulation of the exposed cortex in animals, it can demonstrate a gross idea of the distribution of the area. Mapping is usually done by stimulating with the coil over points in a grid marked on the scalp and recording the amplitude of MEPs that are evoked at each point (Wassermann et al., 1993; Wassermann et al., 1996). The absolute size of the map area for any one muscle depends on the stimulus intensity, and therefore is not a useful variable (Thickbroom et al., 1998). However, the technique is still useful as a way of documenting the centre of gravity of the corticospinal projection since this does not vary with stimulus intensity. For example, the mapping technique has been used to show that prolonged afferent stimulation can shift the centre of gravity of the stimulated muscle, suggesting that there has been a change in the distribution of excitability within the corticospinal projection to the target muscle (Ridding et al., 2001).

1.2.5 Neuroimaging techniques

Neuroimaging techniques by measuring the blood flow, metabolic rate, or particular receptors in the brain can provide observations on changes induced by plasticity in many areas of the brain, rather than being limited to the motor cortex like the MEP. These techniques can also give us some information about the connectivity between brain areas using statistical measures of correlation. The most commonly used methods include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and electroencephalography (EEG) and magnetoencephalography (MEG).

1.2.5.1 Positron emission tomography (PET)

PET is a powerful imaging technique which enables in vivo examination of brain functions. It allows quantification of cerebral blood flow, metabolism, and receptor binding. PET needs a radioactive tracer introduced into the human body to scan the brain. A tracer is essentially a biological compound of interest labelled with a positron emitting isotope, such as ^{11}C, ^{18}F, and ^{15}O. The tracer that is chosen determines what the PET can do. For example, using [^{11}C] raclopride as a tracer, PET scan can be used to detect the level of extracellular dopamine (Endres et al., 1997). $H_2{}^{15}O$-PET can detect the blood flow, while [^{18}F]FDG-PET is for measuring alterations in the regional cerebral metabolic rate of glucose. One of the benefits of PET over fMRI is that it is not usually necessary to perform a certain task during the scan. This make it is possible to detect the brain function 'at rest'.

1.2.5.2 Functional MRI (fMRI)

Functional MRI is a mapping technology that uses blood oxygenation level dependent (BOLD) contrast that can detect the changes in the ratio of oxy/deoxyhaemoglobin that result from neural activity. It has a superior spatial and temporal resolution to PET. Since it detects the changes in BOLD signal, it is necessary to perform a task to 'activate' the brain. The task designed for performing in the scanner is crucial for the function to be explored. There is no need to give any radioactive substance into the human body.

1.2.5.3 EEG and MEG

EEG and MEG are the remaining functional mapping techniques that can be used to

detect lasting changes in brain activation that may accompany plastic changes in synaptic organisation. Advanced EEG methods make it possible to eliminate the contribution of volume currents, in order to obtain reference-free recordings and to disentangle locoregional, rhythmic or transient neuronal activities produced by both tangential and radially orientated generators from discrete brain areas underlying the exploring electrode (Nunez et al., 1997). MEG can localise and measure the intracellular currents in a shallow brain region exactly below the recording sensors without any contribution from volume currents, but is only sensitive to the tangential component of the active dipoles (Romani, 1990). By measuring the change of the cortical activities, in both the temporal and frequency domains, the plasticity effect can be evaluated. However, these encephalographic technologies are not suitable for exploring the function of deep sites of the brain.

1.3 INDUCTION OF PLASTICITY IN CONSCIOUS HUMAN BRAIN

Plastic changes in brain organisation occur naturally throughout life during growth and development and during learning. Recent neurophysiological studies have focussed on the changes in excitability that occurs during learning in adults. For example, it has been demonstrated that the excitability of the corticospinal projection to muscles of the 'reading' finger is increased in Braille readers (Pascual-Leone et al., 1993). Even short periods of practice can lead to changes in excitability in the motor system. Classen et al. (1998) have demonstrated that after subjects had been trained for 30 min to move their thumb voluntarily in the direction opposite to that evoked by a single TMS pulse, the direction of TMS-evoked movements changed, towards the direction of training. This use-dependent plasticity is NMDA and GABA

dependent (Butefisch et al., 2000). Plastic changes are also induced by many pathological conditions, e.g. stroke (Caramia et al., 2000; Trompetto et al., 2000; Alagona et al., 2001; Hallett, 2001), amputation (Schwenkreis et al., 2003), and many other central or peripheral injuries.

Plasticity can also be induced through external stimulation. The sections below describe some of the techniques that have been developed to produce plasticity or plasticity-like effects in the human brain.

1.3.1 Direct current (DC) stimulation

DC stimulation was used quite successfully in the 1960s to induce long lasting changes in cortical excitability in animal preparations and was thought to work by changing the tonic levels of activity in cortical circuits. Recent work has shown that similar phenomena can be induced in the human brain by applying weak direct current (1mA or so) through the scalp for seconds to minutes (Nitsche and Paulus, 2000). The change can outlast by the stimulation for minutes or even hours. The after-effect lasts longer when the stimulus intensity is higher or the duration of stimulation is longer, at least within certain safety limits (Nitsche et al., 2003). The direction of the change of the motor cortex excitability depends on the polarity of stimulation. Stimulation with the positive electrode above the motor area (anodal stimulation), increased the excitability, while the cathodal stimulation decreased the excitability. It has been suggested by pharmacological manipulations that these changes of excitability rely on alternations of the resting membrane potential caused by DC stimulation (Liebetanz et al., 2002).

1.3.2 Repetitive afferent stimulation

Hamdy et al. (1998) found that the excitability of swallowing motor pathways from motor cortex is modulated in a site-specific manner by trains of sensory stimuli at frequencies varying from 0.2 to 10 Hz. Prolonged, repetitive mixed nerve stimulation (500 ms on-500 ms off, 10 Hz, for 2 hours) of the ulnar nerve also leads to a change in excitability of primary motor cortex in normal human subjects (Ridding et al., 2000). The amplitude of MEPs was significantly enlarged and lasted at least 30-40 minutes after the end of the two-hour period of peripheral stimulation (Ridding et al., 2001). MEPs were still larger 24 hours later, although it was not statistically significant. The increase in the MEP size is specific to the muscles innervated by the nerve stimulated and is believed to be due to the change in excitability of cortical connections (Ridding et al., 2001; Kaelin-Lang et al., 2002). Not only the level of excitability, but also its somatotopic distribution in the motor cortex changed after stimulation (Ridding et al., 2001). The effect could be blocked by lorazepam, a $GABA_A$ receptor agonist, indicating that the facilitation induced by prolonged repetitive afferent stimulation involves decreasing cortical GABAergic inhibition (Kaelin-Lang et al., 2002).

1.3.3 Paired associative stimulation (PAS)

Paired stimulation protocols have been used in the motor cortex to induce LTP in animal preparations (Baranyi and Feher, 1981; Hess and Donoghue, 1994; Hess et al., 1996). In humans, paired associative stimulation (PAS), by pairing low frequency stimulation of peripheral afferent nerves with TMS on the motor cortex, has also been demonstrated as a useful paradigm of changing excitability in motor cortex

(Stefan et al., 2000). The stimulation frequency used initially by Stefan et al (2000) was 0.05 Hz, but successful effects can be seen at 0.25 Hz (Ziemann et al., 2004). After stimulating for a period of 30 min, the after-effect may outlast the stimulation by one hour or even longer. The timing interval between the peripheral and TMS stimulation is crucial for the effect on the motor cortical excitability. When the peripheral stimulation was given 25 ms before TMS, cortical excitability was enhanced, while excitability was reduced when the peripheral stimulation preceded TMS by 10 ms (Wolters et al., 2003). The changes caused by PAS are somatotopically specific (Stefan et al., 2000; Ridding and Uy, 2003). Only the MEPs of muscles innervated by the nerve stimulated were changed. Little is known about the mechanism involved. Dextromethorphan, an NMDA receptor antagonist, can block the facilitatory effect of PAS, suggesting the mechanism may be similar to the mechanism of LTP induced in animal studies (Stefan et al., 2002). Theoretically PAS could be used to induce plasticity in any area in the brain only if pre- and postsynaptic stimuli can be delivered to the synapses in a special temporal order. However, only this sensorimotor protocol has been well developed.

1.3.4 Plasticity induction using repetitive TMS

Repetitive stimulation has been shown to be one of the most efficient paradigms to induce long-term plasticity in animal preparations. The development of repetitive TMS (rTMS), as a technique for non-invasive and painless stimulation of the human brain, led to the expectation that similar, potentially therapeutic effects could be produced in conscious humans. Most rTMS stimulators produce an oscillatory magnetic field and generate so called biphasic waveforms, which are different in the

pattern of cortical activation from monophasic waveforms that are generated by most monopulse TMS stimulators. Because the maximal charge transfer happens in the swing between two phases, biphasic stimulus pulses are more efficient in stimulating the brain than monophasic pulses (Maccabee et al., 1998; Kammer et al., 2001).

The first applications of rTMS were, however, not in neural plasticity. In fact, the first report used rTMS to produce speech arrest in order to replace the intracarotid amobarbital (Wada) test to determine speech lateralization (Pascual-Leone et al., 1991). In this instance, rTMS was thought to cause functional disruption of the stimulated site, a phenomenon later referred to as a "virtual lesion" (Jahanshahi and Rothwell, 2000). Repetitive TMS has also been used as a tool to induce seizures in epileptic patients in order to localise epileptogenic foci (Jennum and Winkel, 1994).

Later studies focussed on the potential of rTMS to induce plasticity in brain circuits. In 1994, Pascual-Leone et al. (Pascual-Leone et al., 1994) demonstrated not only a progressive increase in the amplitude of MEPs during a train of rTMS at frequency of 5 or more Hz, but also a facilitatory effect on MEPs that outlasted the train by 3-4 minutes. Later work has extended these observations in a variety of ways.

Frequency, intensity and duration of stimulation are the three main factors that govern the effect of rTMS. A longer duration of stimulation can produce longer and

probably stronger after-effects (Maeda et al., 2000, 2000; Touge et al., 2001). When the intensity is too low, stimulation will not be able to produce any after-effect (Fitzgerald et al., 2002; Huang and Rothwell, 2004). Above a certain threshold, stimulation at lower intensity tends to produce suppression, while a stronger stimulation tends to produce facilitation (Modugno et al., 2001). Indeed, in animal preparations, a LTP induction protocol at a high intensity could actually reverse the LTP induced by the same protocol at a lower intensity (Barr et al., 1995). Frequency is perhaps the most commonly used method of controlling the after effects of rTMS. Low frequency rTMS, usually given at around 1 Hz, can lead to a lasting reduction in cortical excitability (Chen et al., 1997; Maeda et al., 2000; Touge et al., 2001), whereas high frequency (5 Hz or more) rTMS usually increases cortical excitability (Berardelli et al., 1998; Fierro et al., 2001). These definitions of low and high frequency are slightly different from the definitions used by those who work in animal preparations. Here, stimulation at 5 Hz is seen as a low frequency stimulation that leads to LTD. Due to safety concerns, most work with rTMS has used relatively low frequency stimulation as compared with those used in animal preparations.

1.3.4.1 High frequency rTMS

Early rTMS studies focused on the effect of a short train of high frequency stimulation at suprathreshold intensities. A 10-pulse train at 20 Hz and an intensity of 150% RMT over the primary motor cortex facilitated MEPs for 3-4 minutes after the train (Pascual-Leone et al., 1994). Facilitation after a 20-pulse train at 5 Hz and an intensity of 120% RMT lasted for 900 ms, and facilitation after a 30-pulse train at 15 Hz and an intensity of 120% RMT for 30 seconds (Wu et al., 2000). However, in none of these experiments did the after effect last more than 5 min. Few other studies

have been performed with high frequency suprathreshold rTMS. This is mainly because of safety considerations, but there is also the practical consideration of over-heating of the coil during stimulus application.

Lower intensities of high frequency rTMS have been explored more recently. Twelve 150-pulse trains (1800 pulse in total) at 5 Hz and an intensity of 90 % RMT at inter-train intervals of 10 seconds facilitated MEPs for more than 15 min (Peinemann et al., 2004). Other than an effect on the amplitude of MEP, a train of rTMS at 5 Hz also decreases the amount of SICI (Peinemann et al., 2000; Di Lazzaro et al., 2002; Peinemann et al., 2004).

1.3.4.2 Low frequency rTMS

A longer train with larger number of pulses is usually needed to produce lasting effects with low frequency rTMS. Effects seem to depend on the number of pulses and the stimulus intensity. Initial experiments showed that a 20-pulse train, which increased MEP size when the train was given at a frequency of 5 Hz or more, did not show any effect on MEPs when it was given at 1 Hz (Pascual-Leone et al., 1994). However, a 15-min train of 0.9 Hz at 115% of RMT suppressed the MEP for at least 15 minutes (Chen et al., 1997). A train of the same duration at a frequency of 1 Hz and 85% RMT had no effect (Fitzgerald et al., 2002), indicating the importance of stimulus intensity as well as number of pulses.

Even larger pulse numbers have been used by some authors. A train of 1Hz rTMS for 25 minutes and at an intensity of 95% of RMT reduced MEPs for around 30 minutes after the end of the train (Touge et al., 2001). When the intensity was

further lowered to 90% of AMT, a 20-min train at 1 Hz over primary motor area produced no visible effect on MEPs (Gerschlager et al., 2001). In addition to MEPs, other parameters are affected after rTMS: as seen after 5 Hz rTMS, SICI was decreased after a 15-min train of 1 Hz rTMS at 90% RMT (Modugno et al., 2003). SEPs (N20p-P25 and P25-N33) were also suppressed after 1 Hz rTMS over ipsilateral primary motor cortex (Enomoto et al., 2001). When the frequency was lowered to 0.1 Hz, a train of rTMS produced no change on MEP size. However, the amplitude of MEPs were facilitated when this very low frequency stimulation was given when afferent nerves were blocked (Ziemann et al., 1998; Ziemann et al., 1998).

1.3.5 Exploring rTMS-induced plasticity using neuroimaging techniques

A small number of studies have explored the after effects of rTMS with neuroimaging methods. A [^{18}F]FDG-PET study showed increased glucose metabolism of bilateral primary motor areas and left SMA after subthreshold 5-Hz rTMS over left primary motor area, indicating that the effects of rTMS are not limited solely to the site of stimulation (Siebner et al., 2000). Similarly, 1-Hz rTMS over left primary motor cortex resulted in widespread bilateral decreases in rCBF measured with $H_2{}^{15}O$-PET that lasted for at least one hour after stimulation in prefrontal, premotor, primary motor cortex and left putamen (Siebner et al., 2003). A functional MRI (fMRI) study (Lee et al., 2003) confirmed the widespread changes induced by subthreshold 1-Hz rTMS over primary motor area. The same study also showed that the contralateral premotor area and the unaffected primary sensorimotor area increased their activity to compensate for the suppressive effect of the rTMS.

Finally, endogenous dopamine release in ipsilateral caudate nucleus has been induced by 10-Hz rTMS over prefrontal cortex (Strafella et al., 2001) and in ipsilateral putamen by 10-Hz or 5-Hz rTMS over primary motor cortex (Strafella et al., 2003; Ohnishi et al., 2004) by [^{11}C] raclopride PET.

1.3.6 Behavioural effects of rTMS on the motor system

Although rTMS can modify the excitability of the motor cortex or many other regions in the brain in electrophysiological tests or functional images, it seems to be more difficult to show the behavioural consequences on the motor system. It is possible that our brains are so well organised and networked that it is difficult to break the balance and show a behavioural effect. On the other hand, normalisation of the after-effect of rTMS by activating the target muscle (Touge et al., 2001) could also contribute to this difficulty. Most work evaluating behavioural effects has been done following low frequency rTMS. Most studies confirm that there is no effect of 1 Hz motor cortex rTMS on simple motor tasks, e.g. finger tapping speed or maximal and mean peak force and peak accelerations of finger movements (Chen et al., 1997; Muellbacher et al., 2000). Muellbacher et al. (Muellbacher et al., 2002) also reported that the retention of behavioural improvement, but not the performance of other basic or well practiced motor tasks or recall of the newly acquired motor skill, was disrupted by low frequency rTMS on the motor cortex. More complicated tasks, such as the serial reaction time task (Pascual-Leone et al., 1999) may however be affected by rTMS. Twenty minutes 1 Hz rTMS on the motor or premotor area subtly slowed the reaction time in a visual cued choice reaction time task (Schlaghecken et al., 2003). A 10 min train of 1 Hz rTMS on the motor cortex improved ipsilateral sequential simple finger movements (Kobayashi et al., 2004).

1.4 THERAPEUTIC EFFECT OF RTMS

The most attractive reason for studying rTMS is its potential therapeutic application. Inherited from repetitive electrical stimulation in animal preparation, rTMS offers a non-invasive method of inducing plasticity in conscious humans that could be used to treat brain diseases due to hyper- or hypoexcitability in the brain.

1.4.1 Therapeutic effect in psychiatric disorders

The therapeutic effect of rTMS has been studied most thoroughly in patients with major depression. The location most commonly stimulated is the dorsal lateral prefrontal cortex (DLPFC). People choose it because the DLPFC is a TMS accessible region that is within the network related to depression and is highly connected with other nodes in this network (Wassermann and Lisanby, 2001). To stimulate the DLFPC, TMS is usually delivered to the site 5 cm anterior to the optimal place for producing the largest MEP in a hand muscle (hot-spot) (Pascual-Leone et al., 1996). Although high frequency rTMS has been reported to benefit medication-resistant depressed patients (Pascual-Leone et al., 1996; George et al., 2000), some studies suggest that low frequency rTMS may be more effective (Klein et al., 1999; Padberg et al., 1999). There was no particular reason in physiological aspects for them to try low frequency in place of high frequency stimulation. The main reason for this choice of protocol appears to be for safety reasons. However, the results are variable. Hausmann et al. (2004) reported no benefit of rTMS as an "add-on" therapy in depression and although a meta-analysis of controlled studies indicated a fairly robust effect of rTMS (both high and low frequency) the effect was relatively small (Burt et al., 2002)

Initially the idea of using rTMS in depression was to replace electroconvulsive therapy (ECT), which is successful in treating psychotic depression. According to the literature, although rTMS may have equal efficiency in non-psychotic patients, ECT is still more effective in treating patients with psychotic depression (Wassermann and Lisanby, 2001).

Other than studies in depression, a non-blind study using 20 Hz rTMS over the prefrontal cortex found significant improvement in obsessive-compulsive disorder (Greenberg et al., 1997). Another study using lower frequency rTMS over the same area, however, did not show any benefit (Alonso et al., 2001). Functional image studies do not suggest the dorsolateral prefrontal as the most affected area in obsessive-compulsive disorder. This could be the reason why the rTMS was not very effective. Recent trials on patients with schizophrenia using 1Hz stimulation were also equivocal (Wassermann and Lisanby, 2001).

1.4.2 Therapeutic effect in movement disorders

Pascual-Leone (1994) was the first to investigate the possible therapeutic effect of rTMS on patients with Parkinson's disease (PD). He applied 5 Hz rTMS at 90% of RMT to the motor cortex and speeded the performance of the conditioned hand. However a later study by Ghabra et al (1999) failed to replicate this finding. Tergau et al (199a) also reported no clinical benefit using 500 pulses of rTMS at 1, 5, 10 and 20 Hz applied to motor cortex. In another set of studies, although regional cerebral blood flow was reduced by 0.2 Hz rTMS, there was no clinical effect on bradykinesia (Ikeguchi et al., 2003; Okabe et al., 2003).

However, not all studies on Parkinson's disease have been negative. A sham-controlled investigation reported improvement in the bradykinesia of PD patients after trains of 2250 pulses of rTMS at 5Hz and 90% RMT divided into 15 blocks with 10-second pauses in between over hand motor area (Siebner et al., 1999) or 2000 pulses of 5 Hz rTMS at 120% RMT over hand and leg areas (Khedr et al., 2003).

Low frequency (1 Hz) rTMS at subthreshold intensity has been used as a potential treatment in dystonia. Not only was abnormal SICI increased towards normal values after rTMS given to the motor cortex (Siebner et al., 1999) and was the third inhibitory phase of abnormal reciprocal normalized by rTMS over premotor cortex (Huang et al., 2004), but also some improvement in handwriting was demonstrated (Siebner et al., 1999).

1.4.3 Therapeutic effect in other diseases

Another potential application of rTMS is to treat patients with epilepsy. The paradigms used were targeted at reducing excessive excitation and used very low frequency. Both 0.3 Hz (Tergau et al., 1999) and 0.5 Hz (Menkes and Gruenthal, 2000) have been reported to lower the frequency of attacks. One case study also showed a reduction of cortical myoclonus-related epileptic activity after 1 Hz rTMS (Rossi et al., 2004). One Hz rTMS may also have some anti-kindling effect in rats (Anschel et al., 2003). However, no sham-controlled study has been reported so far. There are also a number of papers exploring the effect of rTMS, which has usually been applied to motor cortex at a frequency of 10 Hz, for pain relief (Lefaucheur et al., 2004; Lefaucheur et al., 2004; Pleger et al., 2004).

1.5 PURPOSES OF THE THESIS

There can be no doubt that plasticity of synaptic connections is of fundamental importance to our understanding of brain function. Repetitive stimulation in animal preparations can induce LTP and LTD successfully and efficiently, and the development of repetitive transcranial magnetic stimulation, which can stimulate human brain non-invasively, led to the expectation that such effects could be replicated in humans with potential therapeutic benefits. However, the results so far have been disappointing and weak, inconsistent, and inefficient. I therefore tried to develop novel paradigms of rTMS to produce more efficient, controllable, consistent, long-lasting and powerful effects on the human cortex.

There are several possible reasons for the previous disappointing results of rTMS in humans: first, even in animal experiments, it has been difficult to demonstrate LTP/LTD in the cortex of awake and freely moving animals without the use of extended or repeated sessions of stimulation (Trepel and Racine, 1998; Froc et al., 2000). Second, concerns over safety have limited many studies on humans to the use of relatively low frequencies of stimulation of 25 Hz or (usually) below (Wassermann, 1998). Third, only protocols with regular frequency have been tried in rTMS, while successful paradigms in animal studies often use patterned stimulation, for example, TBS. In addition, TMS in humans is relatively non-focal, and therefore cannot be used to target spatially specific neural connections. In response to these problems, I developed TMS paradigms based on TBS patterns: i.e. patterned, high frequency stimulation using relatively low intensities.

The experiments started by probing the effects of a single burst with different lengths at high frequency (i.e. 50 Hz) and different intensities. I then explored the effect of repeated application of such bursts in modified "theta burst" paradigms (TBS). The underlying mechanisms of TBS were investigated and a simple model was devised. The influence of the status of the cortical excitability on plasticity effects produced by TBS was inspected by asking subjects to activate the target muscle at various points in the conditioning process. A clinical application of TBS is demonstrated by showing different levels of the plasticity in patients carrying the DYT1 gene and healthy subjects.

Chapter 2 Experimental Methods

All investigated subjects and methods and procedures used in different experiments are summarised in this chapter. Any other information, whenever required, will then be given in the respective chapter.

2.1 SUBJECTS

2.1.1 Healthy subjects

The number of subjects recruited in experiments of this thesis and their sex and age are described individually in experiments of each chapter. All participants gave their informed consent prior to participation. At the time of the experiments, all subjects were healthy and reported no history of neurological disease (especially, seizure family history or febrile convulsion history) or any psychiatric history. Subjects were excluded if they had a pacemaker, an implanted medication pump, a metal plate in the skull, or metal objects inside the eye or skull (for example after brain surgery or a shrapnel wound) that might interact with the magnetic stimulus. In addition we excluded women who may have been pregnant. The experiments were performed with the approval of the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery.

2.1.2 Patients with DYT1 dystonia

Familial early-onset primary torsion dystonia is commonly associated with a single

GAG deletion in the DYT1 gene on chromosome 9q34 (McIntosh et al., 1997). The typical phenotype associated with this mutation is of limb-onset dystonia in childhood or early teens, with subsequent progression to generalized dystonia in most cases (Bressman et al., 1994). Despite an autosomal dominant inheritance, the phenotypic penetrance is low: only 30-40% of gene carriers go on to develop dystonia. The penetrance is also age dependent, with the manifestation of symptoms in gene carriers almost always occurring before the age of 25 years. Inter- and intra-familial phenotypic variability is common, with some manifesting gene carriers having only mild focal dystonia, and others being severely affected (Bressman et al., 1994; Opal et al., 2002).

Edwards and Huang et al. (2003) have shown that non-manifesting carriers of the DYT1 gene, although they are clinically unaffected by dystonia, demonstrate some, but not all of the electrophysiological abnormalities found in DYT1 gene carriers with dystonia. This has two implications: first, that the electrophysiological changes previously found in those with other forms of dystonia are not merely an artefact of dystonic movements themselves, as they can occur independently of clinical dystonia. Secondly, it implies that additional abnormalities are needed to cause clinical dystonia, perhaps in sensorimotor integration or basal ganglia ± brainstem outflow. These findings underline the importance of looking outside cortical motor abnormalities in dystonia to other aspects of the motor system for the clues to the genesis of dystonia in DYT1 gene carriers, and those with other forms of primary dystonia. In addition, it is also important to identify potential environmental and genetic modifying factors that could influence penetrance of the DYT1 phenotype.

We recruited 8 DYT1 gene carriers with manifesting clinical dystonia (M-DYT1) from the movement disorder clinics at the National Hospital for Neurology and Neurosurgery. Inclusion criteria were (i) genetic analysis positive for the typical DYT1 gene mutation; (ii) onset of limb dystonia prior to the age of 25 years with or without subsequent progression; (iii) no other cause for dystonia revealed by investigation, including MRI and blood tests; (iv) no brain, spinal or peripheral nerve surgery for dystonia or other cause in the past; (v) no history of other neurological disease; and (vi) no use of botulinum toxin in the past 4 months. Subjects were permitted to continue their other medications as normal during the study. All patients had clinical dystonia affecting the arm and hand used for electrophysiological testing. Six DYT1 gene carriers without manifesting clinical symptoms (NM-DYT1) were ascertained by genetic and clinical assessment of family members of the M-DYT1 group. Inclusion criteria were (i) genetic analysis positive for the typical DYT1 gene mutation; (ii) clinical absence of dystonia confirmed by personal independent assessment of each patient by two neurologists; (iii) no brain, spinal or peripheral nerve surgery for any cause in the past; (iv) no history of neurological disease; and (v) age over 30 years. The average age of those in the M-DYT1 group was 47 years (SD: 8), in the NM-DYT1 group 50 years (SD: 8). Subjects gave their written informed consent to participate.

2.2 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

According to Ampere's law, an electric current will create a circular magnetic field that is coaxial with the direction of current flow. When the electric current flows in a circular loop of wire, the resulting magnetic field is perpendicular to the loop. The

magnetic field in space around an electric current is proportional to the electric current which serves as its source. It should be noted that the current does not have to be time-varying in order to produce the magnetic field. However, for the converse to be true, i.e. for a magnetic field to cause an electric current to flow, then the field must be time-varying. As discovered by Faraday a changing magnetic field induces electrical eddy currents in any conductive object around it. To stimulate the brain, the magnetic stimulator drives a very short current pulse (approximately 200 μs) with a peak amplitude of up to 8,000 A through an induction coil placed over the scalp. The current pulse in the coil, therefore, creates a time-varying magnetic field perpendicular to the coil. The magnetic field penetrates through the skull and then induces eddy current parallel to the coil in the brain. It is the induced current that stimulates cortical tissue and not the magnetic field.

2.2.1 Coil and the site of stimulation

Generally speaking, magnetic stimulation doesn't stimulate a small and predictable site, because it is difficult to restrict the magnetic field to a small volume of tissue. With a circular coil, the induced current is maximal in an annulus under the coil. The larger the coil, the larger the annulus of current, and the less focal the site of stimulation. In addition, the size of the coil influences the depth of penetration of the field in the brain. The larger the coil, the less rapidly the magnetic field falls off with distance from the coil. The focality of stimulation can be changed by altering the geometry of the coil. For example, the electric current induced by coils wound in a figure of eight shape is approximately twice as strong under the junction region of the two loops as it is at each edge.

The amount of neural tissue in the brain that is excited in the brain is influenced by properties of the stimulating pulse, including its intensity, amplitude, waveform and duration (Jalinous, 1991; Brasil-Neto et al., 1992). The skull shape and thickness, and cortical anatomy, especially the orientation of the stimulated neurones (Rothwell, 1997), the coil-tissue distance (Jalinous, 1991), and the temperature of the conducting medium (Maccabee et al., 1998) all affect which neural populations are excited by a stimulus.

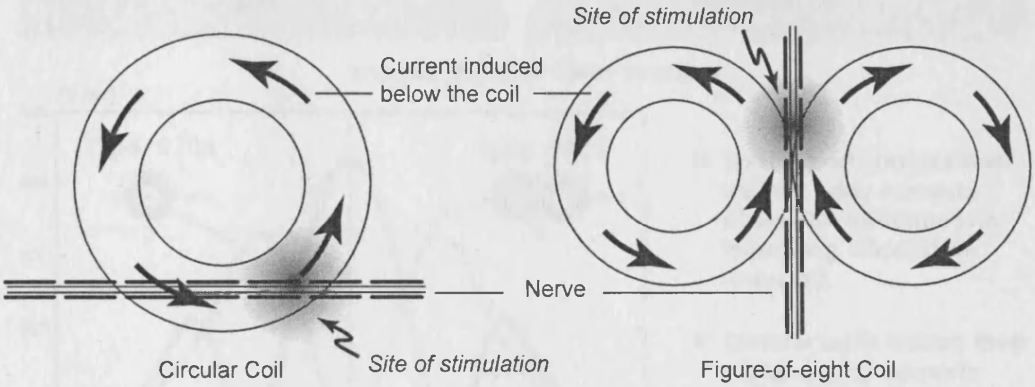

Fig 2.1 ***Site of coil stimulation****. The nerve can only be triggered when current cuts through its membrane.*

It is important to note that the stimulus must cause a transmembrane current to flow in order to activate a nerve fibre. When a nerve is parallel to an electrical field, the current only flows inside the nerve without cutting through the membrane and hence an action potential will not be triggered. Take the circular coil for example: if an axon lies circularly just beneath the circle of the coil, it will not be stimulated. Only if the axon runs out of the circle and cuts the electrical field, will a virtual anode-cathode pair be created across the membrane to activate the axon (Fig 2.1). It is important to remember that the stimulation site is not underneath the centre of a circular coil. In fact this is where stimulation is least likely to occur. The

figure-of-eight coil, in which two circular coils are placed side by side, induces an electrical current under the junction region that is twice as large as elsewhere under the two rings (Fig 2.2). Thus if an axon runs parallel to the current along the junction, stimulation actually happens at where the junction region divides into two branches.

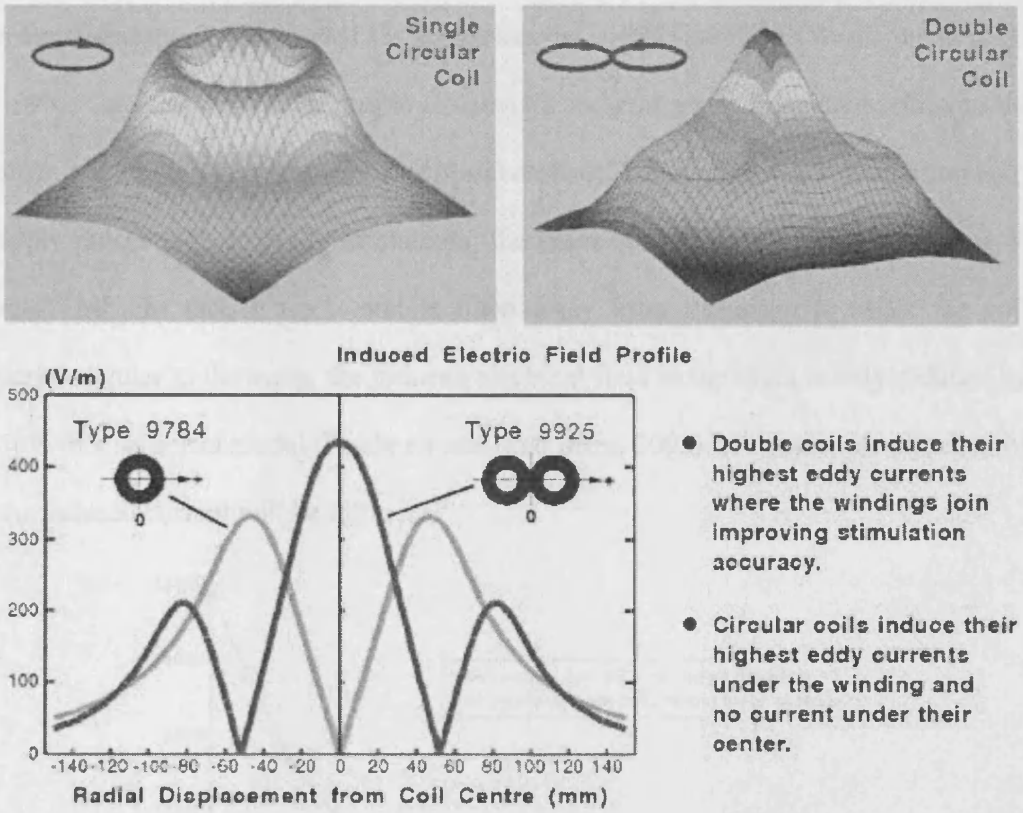

Fig 2.2 ***The induced electric field profile of circular and figure-of-eight coils differ widely because of their geometry.*** *There is no induced electric field directly under the centre of a circular coil. The electric field reaches maximum approximately under the mean diameter. In the case of figure-of eight coil, it is at a maximum under the junction of the two coils and has two smaller characteristic peaks on either side.*

The depth of penetration of a magnetic stimulation depends on coil size, coil shape, intensity of the applied stimulation, and the anatomical factors. A simulation using a homogeneous model with straight nerves showed the depth of penetration is pretty much the same in a circular coil and a figure-of eight coil, although it is slightly deeper with a figure-of-eight coil (Fig 2.3) (Barker, 2002). TMS activation is

supposed to take place at the maximum point of the induced electrical field. The locus of the maximum electrical field could be affected by factors like the electrical properties of the underlying neural tissue. For example, in sensory-motor cortex, the maximum electrical field in TMS has been found to be within 10-20 mm by using magnetoencephalography (MEG) (Morioka et al., 1995) and PET (Wassermann et al., 1996). On the other hand, people usually tilt the coil away from the surface to do sham stimulation to control for any placebo effect. The aim of sham stimulation is to apply pulses without really stimulating the brain but still causing the perception of real TMS. In fact, when a coil is tilted away from the scalp to make the coil perpendicular to the scalp, the induced electrical field in the brain is only reduced by 50% in a spherical model (Ruohnen and Ilmoniemi, 2002). However, the direction of the induced current will be different.

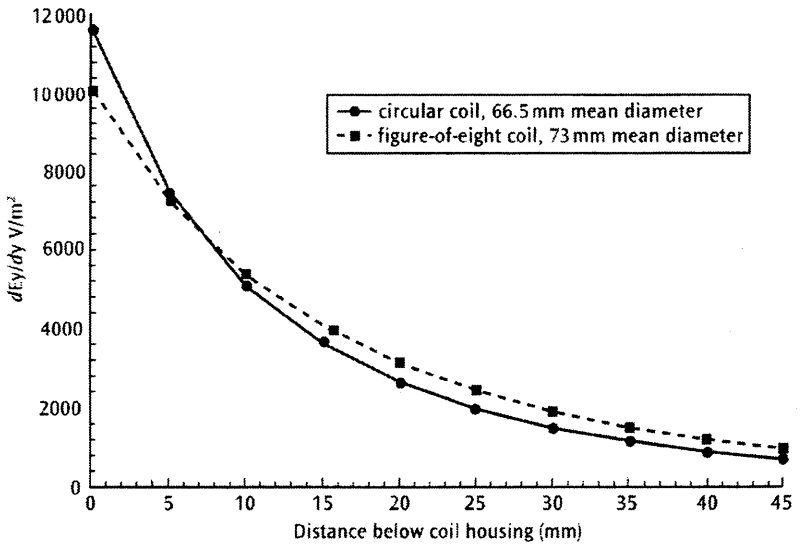

Fig 2.3 ***Electric field and the distance below a circular coil.*** *The decrease of the spatial derivative of electric field is not very different with depth below a circular coil and a figure-of-eight coil.*

Magnetic stimulation in this thesis was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK)

connected directly to a Magstim 200 machine (Magstim Co., UK) or though a Y-cable to two Magstim 200 machines.

2.2.2 Hot spot and motor threshold

In all TMS experiments in this thesis, the optimal location of the coil (the "motor hot-spot") was determined by the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral target muscle when the subject was relaxed.

Motor threshold is a measure of cortical excitability and is the lowest intensity of TMS at which a MEP of a defined size can be recorded in the target muscle. According the guidelines of the International Federation of Clinical Neurophysiology (Rothwell et al., 1999), we defined the resting motor threshold (RMT) as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 50μV in five out of ten trials. The active motor threshold (AMT) was defined as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 200μV in five out of ten trials during a voluntary contraction at a low level (10-20 % of the maximum voluntary contraction) of the contralateral target muscle. In fact, this is not perhaps the best definition. Mills and Nithi (1997) recommended a better approach. These authors defined the motor threshold as the average of the upper threshold, which is the minimum intensity that produces a positive response in all 10 stimuli, and the lower threshold, which is the maximum intensity that produces no response in all stimuli.

Ziemann and colleagues (Ziemann et al., 1995; Ziemann et al., 1996; Ziemann

et al., 1996, 1996) have reported that corticomotor threshold was only changed by the administration of drugs acting on the neuronal membrane excitability, but little effect was noted by giving drugs affecting synaptic transmission. These data suggest that the motor threshold is primarily affected by the membrane properties of the corticospinal system.

2.2.3 Intracortical inhibition and facilitation

The intracortical inhibitory and facilitatory circuits can be tested by the paired pulse technique. The technique was reported independently in two groups in 1992 (Claus et al., 1992; Valls-Sole et al., 1992) using two pulses at a suprathreshold intensities. This technique is now mainly used to test the long-interval intracortical inhibition (LICI), which happens when the interstimulus interval (ISI) of the two pulses ranges between 50 to 200 ms.

Another paired pulse paradigm (Kujirai et al., 1993) using subthreshold conditioning pulse and a suprathreshold test pulse to test the intracortical inhibitory and facilitatory circuit in the motor cortex is more commonly used. In this technique, as with the paradigm for LICI, two TMS stimuli are delivered to the primary motor cortex through a single coil. However, the conditioning pulse is at a subthreshold intensity. The amount of inhibition/facilitation depends on the intensity of the conditioning stimulus. In the past, different groups have tended to use different intensities. For example, Kujirai and colleagues (Kujirai et al., 1993) used 80% of RMT , while others have used 5% below AMT ((Ziemann et al., 1996; Di Lazzaro et al., 1998) as the intensity. The intensity of the test pulse is usually adjusted to produce an MEP of about 1 mV in peak-to-peak amplitude in the target muscle, so

that the test MEP is big enough to show good inhibition, but is not too big to have ceiling effect after facilitation. The ISIs used in this paradigm range from 1 ms to 20 ms. When the ISI is at 1-5 ms, the test MEP will be suppressed by the conditioning pulse through the inhibitory circuit. The test MEP will be enhanced through the facilitatory circuits, when the ISI is at 8-20 ms.

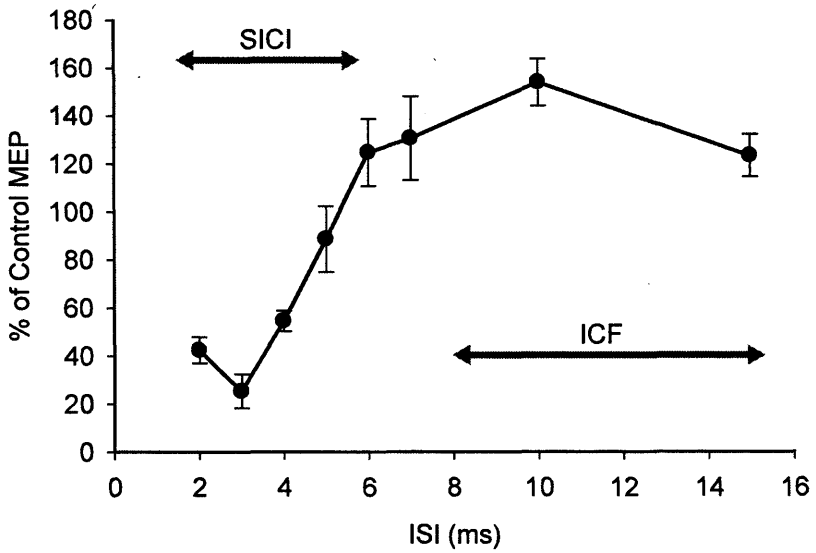

Fig 2.4 ***A normal curve of intracortical inhibition and facilitation*** *tested with the paradigm first described by Kujirai et al. The conditioning intensity used in this figure was 80% AMT, and the test intensity was the intensity to produce 1mV peak-to peak amplitude when the test pulse was given alone. This figure represents group data. Error bars refer to the standard error of the measurements.*

Fig 2.4 shows typical inhibition and facilitation curve results in the paired pulse paradigm using 80% AMT as a conditioning intensity. The effect of the intensity of the conditioning pulse on the inhibition and facilitation has been explored in a few studies (Kujirai et al., 1993; Ziemann et al., 1996). The inhibition at the ISI of 3 ms began when the intensity of the conditioning pulse was 60% RMT and is increased as the intensity was turned up. The inhibitory effect reached the maximum when an intensity of 80% RMT or 90% AMT was used. Increasing the conditioning intensity

even further resulted in less inhibition. The intensity for producing facilitation is less well known. It is suggested that the threshold for producing facilitation effects is 90% RMT (Kujirai et al., 1993) or 80% AMT (Ziemann et al., 1996). However, The variability was high if a single conditioning stimulus intensity was used to compare the percent intracortical inhibition or facilitation between subjects, or between sessions (Orth et al., 2003). These authors suggest that the ratio of conditioning stimulus intensity: AMT is a robust and useful additional measure of the integrity of neuronal circuits underlying intracortical inhibition/facilitation.

The inhibition induced in this paired pulse paradigm at ISI of 1-5 ms is called short-interval intracortical inhibition (SICI), whereas the facilitation induced at ISI of 8-20 ms is called intracortical facilitation (ICF). GABA agonists and NMDA receptor blockers both enhanced the SICI and decreased ICF. Dopamine receptor agonists also have the similar effects, while haloperidol (D2, a dopamine receptor, antagonist) decreased SICI and enhanced ICF.

In the experiments in this thesis, 80% and 90% of AMT were chosen as the conditioning stimulus intensities for producing SICI and ICF respectively, to obtain reasonable amount of inhibition or facilitation. However, when SICI and ICF were tested in the same session, 90% of AMT was used.

2.2.4 Repetitive TMS

Instead of giving a single pulse to probe the cortical excitability in the brain, TMS can be given repetitively to produce repeated stimulation similar to that used in animal preparations for plasticity-induction. Repetitive stimulation equipment is now

available from all major magnetic stimulation manufacturers. Present day commercial repetitive TMS machines are capable of producing a frequency up to 50 Hz, although some custom devices can generate a frequency of 100 Hz or even more. Most repetitive stimulation machines use oscillatory output to produce biphasic discharge currents instead of the monophasic discharge currents commonly used in monopulse machines. Biphasic discharge currents can reuse around 40% of the original energy at the end of the stimulation pulse. Thus only 60% of the original energy is needed for the next pulse, making the machine much more efficient. On the other hand, at the period when the phase changes, the large time-varying difference makes biphasic stimulation capable of producing larger currents than a monophasic stimulation with the same energy. In addition, both phases can activate descending volleys, including direct (D) and later indirect (I) waves, that depend the relative threshold and the relative amplitude of each phase. While using the commonly used figure-of-eight coil, current in a posterior-anterior (PA) direction provokes preferentially I1 waves (the first I wave). It is easier to recruit D waves with current in the brain in a lateral to medial (LM) direction (Di Lazzaro et al., 1998). Anterior-posterior (AP) stimulation seems to be a complex process that may differ between subjects (Di Lazzaro et al., 2001), although it has been suggested that it recruits I3 waves earlier than I1 waves (Sakai et al., 1997). The reversal phase of a hyperpolarising-depolarising bipolar pulse was more effective on an axon than a monophasic depolarising pulse (Maccabee et al., 1998; Di Lazzaro et al., 2001). McRobbie and Foster (1984) found that only 59% of the peak rate of change of magnetic field of a monophasic stimulation is required for a biphasic stimulation to achieve the same level of stimulation. However, biphasic discharge currents produce more heat dissipation in the coil, discharge click noise, and probably less accuracy than monophasic stimulation currents do. The biphasic stimulus also produces more

complex pattern of cortical activation than that monophasic pulses according to cervical epidural recordings (Di Lazzaro et al., 2001).

Most rTMS studies published to date have focussed on relatively low frequencies of stimulation (1-5 Hz, and never more than 25 Hz) due to safety concerns. Although several studies have reported long-lasting after effects of rTMS in conscious humans, the effects have been variable, inconsistent, and weak. The paradigms of rTMS used in the experiments of this thesis are mainly based on high frequency stimulation at a frequency up to 50 Hz. Repetitive magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK) connected to a Magstim Super Rapid Package (Magstim Co., UK) with four boosters. In some experiments we used a special combining module (Magstim Co., UK) to couple two Magstim 200 machines and one Magstim Super Rapid Package (Magstim Co., UK) so that we could test cortical function of the motor cortex with monophasic pulses (or pairs of pulses) immediately after rTMS.

2.3 H-REFLEX

The H-reflex was first described by Hoffmann in 1918, as a late response to submaximal electrical stimulation of the tibial nerve that occurred with a latency similar to the response recorded as a result of a tendon tap. It was later termed the "Hoffmann response" or "H-reflex" by Magladery and McDougal in his honour (Stolp-Smith, 1996). As indicated in Fig 2.6, the H-reflex is primarily a monosynaptic response similar to the tendon tap. However, because the electrical

pulse stimulates afferent fibres in the nerve directly, the H-reflex bypasses the spindle component of the tendon tap. Increasing the stimulus intensity initially causes the amplitude of the H-reflex to increase. However, when the intensity increases further, more and more of the orthodromic reflex volley is blocked by antidromic impulses and reduces the amplitude of the H-reflex. The H-reflex is best elicited by a long duration (1ms) nerve stimulus since this tends to activates afferent fibres more effectively than efferent fibres.

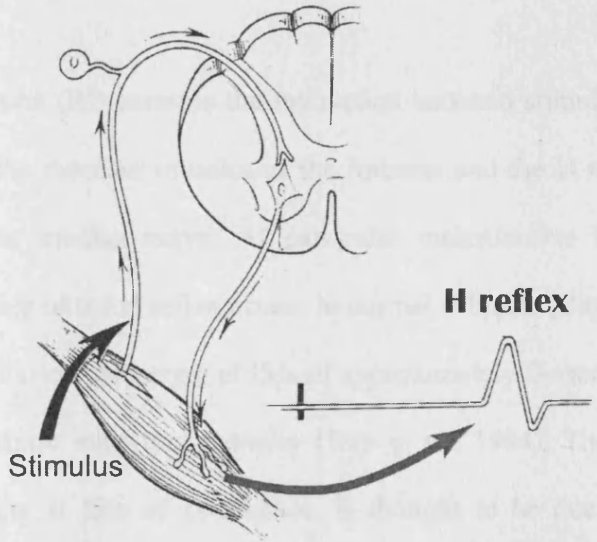

Fig 2.5 ***Physiology of the H reflex.*** *Selective sensory inputs of muscle spindle afferents could evoke muscle responses through a monosynaptic reflex.*

In normal adults, the H-reflex can be recorded reliably from the soleus and flexor carpi radialis (FCR) muscles. Other muscles, like palmaris longus, flexor carpi ulnaris, anterior tibial, vastus medialis, masseter, extensor digitorum communis, and ulnar-innervated hand intrinsic muscles have also been reported to have H-reflexes (Stolp-Smith, 1996). It should be noted that the H-reflex can be enhanced by activating the muscle recorded, and hence it is best to record the response at rest. It is also important to differentiate H-reflex from F wave, which occurs after M response

and reaches maximal amplitude with supramaximal stimulation.

The H-reflex in the experiments of this thesis was obtained from FCR muscle. Electric pulses were supplied by a constant current generator DS7A (Digitimer, Welwyn, UK). The stimuli were delivered in the antecubital fossa to stimulate the median nerve. Stimulation duration was 1000 μsec, and the intensity used was that which produced the maximum size of the H reflex, without producing an M wave.

2.3.1 Reciprocal inhibition

Reciprocal inhibition (RI) assesses the interaction between stimulation of the radial nerve supplying the extensor muscles of the forearm and the H reflex produced by stimulation of the median nerve. At particular interstimulus intervals (ISIs), a reduction in the size of the H reflex occurs in normal subjects (Day et al., 1984). The first phase of inhibition, occurring at ISIs of approximately 0msec, is mediated by a glycinergic disynaptic inhibitory pathway (Day et al., 1984). The second phase of inhibition, occuring at ISIs of 10-20msec, is thought to be due to presynaptic Ia inhibition of afferent fibres that mediate the H reflex (Berardelli et al., 1999). The origin of the third phase of inhibition, occurring at ISIs of 70-500msec, is less well known and might go through the polysynaptic long latency stretch reflex pathway (Tsai et al., 1997).

In the experiments in this thesis, we grouped these ISIs into three phases of RI: one occurring at 0 msec, one at 10 to 20 msec, and one at 70 to 750 msec. Subjects were seated in a comfortable chair. We attached Ag–AgCl electrodes to extensor digitorum communis and to flexor carpi radialis. Electric pulses were supplied by two constant current generators (Digitimer, UK). One electrical stimulator was used

to stimulate the median nerve in the antecubital fossa. Stimulation duration was 1,000 μsec, and the intensity used was that which produced the maximum size of the H reflex, without producing an M wave. The second electrical stimulator was used to stimulate the radial nerve above the elbow. The duration of the stimulus was 500 μsec, and the intensity used was the minimum one that produced an electromyographic (EMG) response of greater than 50 μV from extensor digitorum communis. We recorded H reflex size during stimulation of the median nerve alone and for ISIs of -1, 0, 3, 5, 10, 20, 30, 50, 70, 100, 300, 500, and 750 msec. Stimuli were given in a random order in one block of 60 trials and two blocks of 50 trials. We were sensitive to the possible modulation of H reflex size by muscle contraction; therefore, any trials where EMG movement artefact occurred were rejected online and were repeated.

2.4 ELECTROMYOGRAPHY (EMG) RECORDINGS

Surface EMG recordings were performed with 1-cm-diameter Ag/AgCl-plated surface electrodes placed over the right first dorsal interosseous (FDI), flexor carpi radialis (FCR), or abductor digiti minimi (ADM) muscles using a belly-tendon montage. A Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK) was used to amplify and analogue filter (3 Hz to 2 kHz) raw signals. Signals were recorded at a sampling rate of 5 kHz and stored on a personal computer for later analysis by Signal software (Cambridge Electronic Design Ltd., Cambridge, UK) through a Power 1401 data acquisition interface (Cambridge Electronic Design Ltd., Cambridge, UK).

2.5 DATA ANALYSIS

The peak-to-peak amplitude of each MEP or H-reflex was measured and the mean amplitude of 10 consecutive MEP or H-reflex was automatically calculated, using inhouse software (NuCursor, Sobell Department, Institute of Neurology, University College London).

Data were analysed using SPSS for Windows version 11.0. Repeated measures ANOVA was used to compare effects on series of the time course evaluation (e.g. effects before and after TBS), and pairwise multiple comparisons with Bonferroni correction were used for post hoc tests and also to compare the effect of TBS on H-reflexes and MEPs recorded from FCR. Statistics for the data were performed on absolute amplitude values or normalised amplitudes.

Chapter 3 The effect of a single burst

The purpose of chapter is to explore the effect of applying a short burst of high frequency repetitive transcranial magnetic stimulation (rTMS) to the human motor cortex as a preparatory investigation before attempting theta burst stimulation in humans. 5 or15 pulses of 50Hz rTMS were given at 50-80% active motor threshold (AMT). The time course of changes in MEP size and short interval intracortical inhibition (SICI) were evaluated from 20 to 300 ms after the end of each burst in the relaxed first dorsal interosseous muscle of 15 healthy volunteers. No subjects noted any adverse effects. MEPs were enhanced and SICIs were reduced at 20 ms after a burst of either 5 or 15 pulses at 70 or 80 % AMT, but not at 50% AMT. Subsequent experiments used a 5 pulse burst at 80% AMT. The threshold for producing SICI increased from 60 to 80% AMT when tested 10 or 20 ms after the end of the burst. MEPs were enhanced for 100 ms, whereas SICI was reduced for 200-300ms. In conclusion, a short burst of low intensity 50 Hz rTMS over the hand motor area transiently increases MEP amplitude with a longer lasting decrease in SICI. This means that it may be possible in future experiments to apply theta burst conditioning safely to the human cortex.

3.1 INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) is being used in many centres to produce after effects on cortical excitability that outlast the period of stimulation. Several studies have shown that the effect of rTMS is frequency dependent. Low frequencies of rTMS (1 Hz or less) tend to decrease neuronal excitability (Chen et al.,

1997; Maeda et al., 2000; Gerschlager et al., 2001), whereas high frequencies (5 Hz or more) increase excitability (Berardelli et al., 1998; Maeda et al., 2000). Although the mechanism of these effects is not completely understood, it seems likely that synaptic plasticity plays an important role. Such reasoning has led several groups to explore the possible use of rTMS as a therapeutic tool to induce changes in synaptic function in patients with psychological (Speer et al., 2000; Daskalakis et al., 2002; Dragasevic et al., 2002; Martin et al., 2002) or movement disorders (Siebner et al., 1999; Shimamoto et al., 2001; Wassermann and Lisanby, 2001; Gilio et al., 2002).

All published work using rTMS in humans has been limited to frequencies of 25 Hz or less. However, in animal experiments, powerful effects on synaptic plasticity are often produced by using repeated short (around 4 pulses) bursts of high frequency (50-200 Hz) stimulation given 3-5 times per second (theta burst stimulation) (Hess et al., 1996; Otani et al., 1998; Urban et al., 2002). As a preparatory investigation prior to the introduction of theta burst stimulation in humans, we explored the effect of applying a single short burst of 50 Hz rTMS to the human motor cortex in order to document its effects on corticospinal excitability. Based on the present safety guidelines (Wassermann, 1998), which allow stimulation at 100% of MEP threshold using a frequency of 25 Hz for 1.28 seconds, we decided to use intensities of up to 80% of active motor threshold (AMT) given at 50 Hz with up to 15 pulses per burst.

3.2 METHODS AND SUBJECTS

3.2.1 Subjects

We studied 15 right-handed healthy volunteers (9 men, 6 women; mean age ± SD,

32.4 ± 7.4 years). All participants gave their informed consent prior to participation. The experiments were performed with the approval of the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery.

3.2.2 Electromyographic *(EMG) recordings*

EMGs were performed with 1-cm-diameter Ag/AgCl-plated surface electrodes placed over the right first dorsal interosseous muscle (FDI), using a belly-tendon montage. A Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK) was used to amplify and analogue filter (3 Hz to 2 kHz) raw signals. Signals were recorded at a sampling rate of 5 kHz and stored on a personal computer for later analysis by Signal software (Cambridge Electronic Design Ltd., Cambridge, UK) through a Power 1401 data acquisition interface (Cambridge Electronic Design Ltd., Cambridge, UK). Trials in which the target muscle was not relaxed (as monitored with an EMG gain of x5000) were rejected online, and that stimulus condition was repeated.

3.2.3 Transcranial magnetic stimulation (TMS)

Magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK) connected though a combining module (Magstim Co., UK) to two Magstim 200 machines and one Magstim Super Rapid Package (Magstim Co., UK).

Subjects were seated in a comfortable chair with their eyes open. The coil was

placed tangentially to the scalp with the handle pointing backwards, which is thought to preferentially activate the corticospinal cells trans-synaptically (Kaneko et al., 1996). The optimal location of the coil was determined by the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral FDI when the subject was relaxed (the "motor hot-spot"). According the guidelines of the International Federation of Clinical Neurophysiology (Rothwell et al., 1999), we defined the resting motor threshold (RMT) as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 50μV in five out of ten trials. The active motor threshold (AMT) was defined as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 200μV in five out of ten trials during a voluntary contraction of the contralateral FDI.

The peak-to-peak amplitude of MEPs evoked by a suprathreshold stimulus and short intracortical inhibition (SICI) were used to probe the excitability of the motor cortex. We choose 2 ms as the interstimulus interval (ISI) to evaluate the SICI, which was first described by Kujirai et al (Kujirai et al., 1993). The intensity of the conditioning (first) stimulus was set to 80% of AMT. The test (second) stimulus was set at an intensity that would evoke an EMG response of around 1 mV peak-to-peak amplitude, when given alone.

3.2.4 Experimental protocol

3.2.4.1 Effect of burst length and intensity (Fig 3.1A)

Seven subjects (4 men, 3 women; mean age, 29.3 ± 1.9 years) were recruited for this experiment in which we examined the changes in amplitude of MEP and SICI that

occurred 20 ms after bursts of 50 Hz rTMS with different lengths and different stimulus intensities.

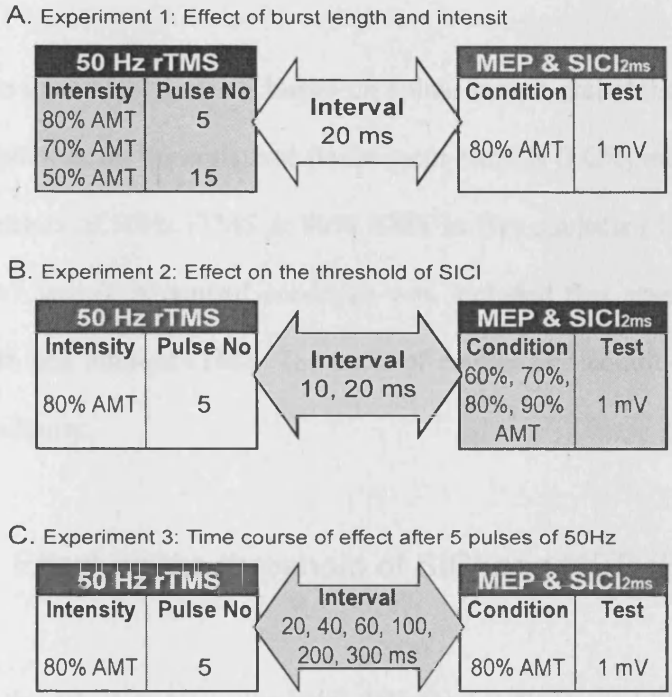

Fig 3.1 ***The experimental protocol.*** *The factors tested in the experiment 1 (A) are the length and intensity of the burst. The intensity of the conditioning pulse of SICI was adjusted to investigate the effect of the burst on the threshold of SICI in experiment 2 (B). Then we evaluated the time course of effect after 5 pulses of 50Hz in experiment 3 (C).*

We delivered 50Hz rTMS at three different stimulus intensities: 50%, 70% and 80% of AMT. Both 5 and 15 pulses of 50Hz rTMS were given at each stimulus intensity. We assessed the effect of these different stimulation conditions on MEP amplitude and SICI 20ms after the end of the rTMS burst. Measures of MEP amplitude and SICI evaluated in the absence of any conditioning pulses are referred to as control. The assessments were separated into three blocks of 60 trials with the different stimulation conditions given at variable intervals (4.5-5.5 sec) in a random sequence. Because rTMS increased the size of the test MEP we also conducted a

series of experiments in 3 subjects where we evaluated SICI after adjusting the intensity of the test stimulus to maintain MEP size at around 1 mV peak-to-peak amplitude after the end of the rTMS.

To assess the affect of rTMS bursts on spinal motor excitability, we tested the MEP and H-reflex in the contralateral flexor carpi radialis (FCR) muscle 20ms after the end of 5 pulses of 50Hz rTMS at 80% AMT in five subjects (3 men, 2 woman; mean age 31±7 years). A control condition was included that assessed MEPs and H-reflexes with and without rTMS. Ten trials of control and conditioned trials were intermixed randomly.

3.2.4.2 Effect on the threshold of SICI and MEP (Fig 3.1B)

We evaluated the effect of a 5 pulse burst delivered at 50 Hz and 80% AMT on the threshold for producing SICI tested at 10 or 20 ms after the end of a burst in five subjects (3 men, 2 women; mean age, 37.0 ± 10.8 years). We tested five blocks using five different intensities of conditioning stimulus: 60%, 70%, 80%, 90%, and 100% AMT. We performed 10 trials of each condition given at variable intervals (4.5-5.5.seconds) in a random sequence. We also checked the influence of a 5 pulse burst on RMT or AMT at 20 ms after the end of the burst in 5 subjects (3 men, 2 women; mean age 31±7 years).

3.2.4.3 Time course of effect after 5 pulses of 50Hz (Fig 3.1C)

Based on the results of the first experiments, we chose to look in more detail at the effects of 5 pulse bursts delivered at 50 Hz and 80% AMT on MEP amplitude and

SICI at intervals of between 20 and 300 ms after the end of each burst. Seven subjects (4 men, 3 women; mean age, 29.7 ± 6.0 years) participated this experiment. MEP amplitude and SICI were assessed at seven time points: 20, 40, 60, 100, 150, 200, and 300 ms, after the end of each burst. We also measured the effect of monophasic single pulses of TMS given at 80% AMT on MEP amplitude and SICI at 20, 150, and 200 ms after the single pulse. We performed 10 trials of each condition, which were given at variable intervals (4.5-5.5.seconds) in a random sequence.

3.2.5 Data analysis

Repeated-measures analysis of variance (ANOVA) was used to analyse group factors. When necessary, *t*-tests were used to compare the individual effects.

3.3 RESULTS

No subject noted any adverse effect during or after the experiments.

3.3.1 Effect of burst length and intensity

In this experiment we investigated how the amplitude of MEPs and the percentage SICI were modulated when test pulses were given 20ms after the end of a burst of 5 or 15 pulses at an intensity of 50%, 70% or 80% AMT. A 20ms interval was selected since this is the time between pulses in a 50 Hz train, and would therefore be the time of the next pulse in a longer burst. Figure 3.2A shows the effect on MEP amplitude. A two way ANOVA comparing the data from the 5 and 15 pulse bursts revealed a

significant main effect of INTENSITY ($F(2,12) = 3.9$, $p < 0.05$), but not of PULSE number. Thus the effect on MEP amplitude was the same for both 5 and 15 pulse bursts. Post hoc comparison of the combined data from the 5 and 15 pulse bursts (not illustrated) with the control MEP amplitude showed that bursts at 70% and 80% AMT increased the amplitude of MEPs by approximately 38% and 48% respectively (paired t tests, $p<0.05$ for both comparisons), but no effect was observed with stimulation at 50% AMT.

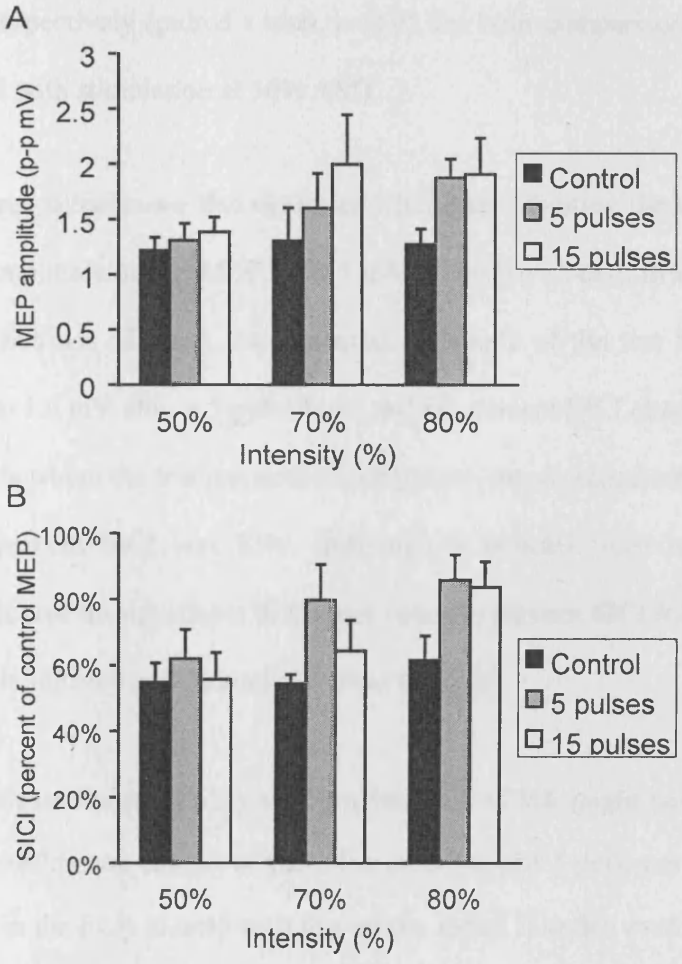

Fig 3.2 ***The effect of bursts of 50Hz rTMS on MEP amplitude (A) and SICI (B).*** *SICI is expressed as a percentage of the unconditioned MEP in each subject. This figure represents group data. Error bars refer to the standard error of the measurements.*

The effects on SICI are shown in figure 3.2B. A two way ANOVA comparing SICI following 5 and 15 pulse bursts with control SICI revealed a significant main effect of INTENSITY ($F(2,12) = 5.4$, $p < 0.05$), but not of PULSE number. Thus, like the MEP, the change in percent SICI was the same after either the 5 or 15 pulse bursts. Post hoc comparison of the combined data from SICI following 5 and 15 pulse bursts (not illustrated) with the control SICI showed that bursts at 70% and 80% AMT decreased the amount of SICI from 58% to 78% control and from 62% to 85% control respectively (paired t tests, $p<0.05$ for both comparisons), but that no effect occurred with stimulation at 50% AMT.

In the three subjects we also evaluated SICI after adjusting the test intensity to maintain the amplitude of test MEP to be 1 mV following a burst. In trials where the test intensity was not adjusted, the averaged amplitude of the test MEP increased from 1.1 mV to 1.6 mV after a 5 pulse burst and the percent SICI changed from 41% to 74%. In trials where the test intensity was adjusted, the averaged test MEP was 1.2 mV and the percent SICI was 83%. Independent samples t-test in each subject individually showed no significant difference between percent SICI following a burst of 5 pulses with adjusted and non-adjusted test intensity.

To investigate the possibility that low intensity rTMS might have some effect on spinal excitability, we compared the effect of a burst of 5 pulses at 80% AMT on MEPs evoked in the FCR muscle with that on the spinal H-reflex evoked in the same muscle. A two factor ANOVA with ± rTMS and RESPONSE TYPE (i.e. H-reflex or MEP) as main factors revealed a significant ± rTMS x RESPONSE TYPE interaction ($F=5.54$, $p<0.05$). Post hoc comparisons showed that this was because MEP size was facilitated by rTMS ($t=-3.29$, $p<0.05$), but that H reflex size was unchanged ($t=0.89$,

ns).

3.3.2 Effect on the threshold of SICI and MEP

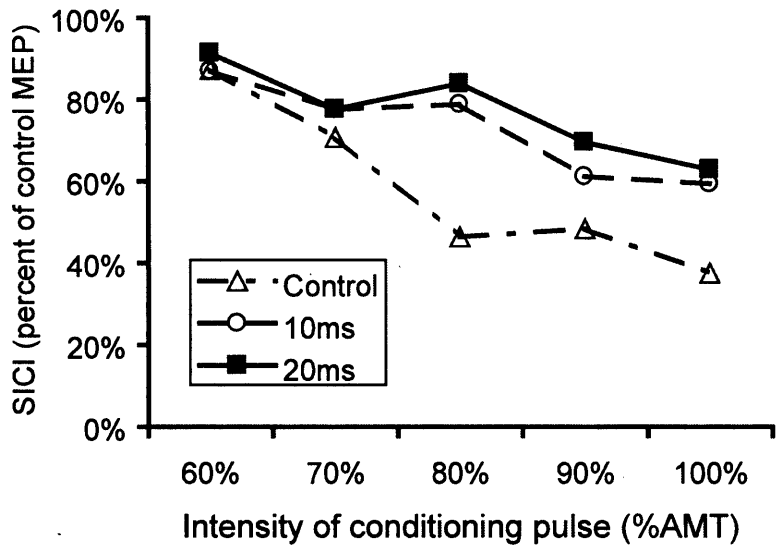

Fig 3.3 ***The effect of a 5 pulse burst at 50Hz and 80% AMT on the amount of SICI using different intensities of conditioning pulse (x axis).*** *SICI is expressed as a percentage of the unconditioned MEP in each subject. This figure represents group data.*

In the experiments above, the percent SICI was reduced at 20ms after a burst of 5 pulses, but interpreting this change is difficult because the MEP was also facilitated at that time. Although SICI is usually more prominent the larger the MEP (Chen et al., 1998), facilitation of the response might also increase the effective amplitude of the conditioning stimulus. Given that there is a "U"-shaped dependence of SICI on conditioning intensity (Kujirai et al, 1993), it is possible that increasing the effectiveness of the conditioning stimulus would decrease the SICI. In order to overcome this problem we investigated whether the threshold for producing SICI was changed by a conditioning burst of 5 pulses at 80% AMT. SICI was tested at both 10 and 20ms after the end of the burst, and five different intensities of conditioning stimulus were used to evoke inhibition: 60%, 70%, 80%, 90%, and

100% AMT. The results are shown in figure 3.3. A 2 way ANOVA with CONDITION (10 ms and 20 ms after a burst and control) and conditioning stimulus INTENSITY revealed a significant 2 way interaction ($F(8,32)=2.7$, $p<0.05$). Follow up 2 way ANOVAs showed that the 10 and 20ms CONDITIONs did not differ from each other but each differed from the control. In a final analysis we combined the data from 10 and 20ms and compared the percent SICI to control values at each INTENSITY of conditioning stimulus. There was significantly more inhibition in the control CONDITION at all intensities of stimulation (paired t test, $p<0.05$ for all comparisons). In addition, in the 10 and 20ms CONDITION, significant inhibition occurred only when the intensity was ≥ 80% AMT. In contrast, in the control CONDITION, inhibition was significant at all intensities studied.

The rest motor threshold was significantly reduced from 53 ± 19% to 46 ± 15% by a burst of 5 pulses at 80% AMT ($t=3.30$, $p<0.05$), whereas the active motor threshold was not significantly changed ($t=0.34$, ns).

3.3.3 Time course of effect after 5 pulses of 50Hz

These experiments were carried out with a burst of 5 pulses of 50Hz rTMS at an intensity of 80% AMT or a single monophasic pulse at the same intensity. Test MEPs and percent SICI were evaluated between 20 and 300ms after the end of the burst/single pulse, and the results were compared. Figure 3.4A shows the time course of the effect on the MEP amplitude measured between 20 ms to 300 ms after either a 5 pulse burst or a single pulse at 80% AMT. We first performed a two factor ANOVA with TIME and PULSE number as main factors on the data at the common ISIs. There was a significant interaction between TIME and PULSE number ($F(1, 12)=3.5$,

$p<0.05$). Subsequent paired t tests at these time intervals showed that this was because at 20ms, MEPs were facilitated after the burst but not after a single pulse. Follow up one-way ANOVAs on all time points showed that the MEP amplitude was affected by ISI ($F(7,42)=3.1$, $p<0.05$) after a 5 pulse burst, but was not changed by a single pulse at any ISI ($F(3,18)=0.65$).

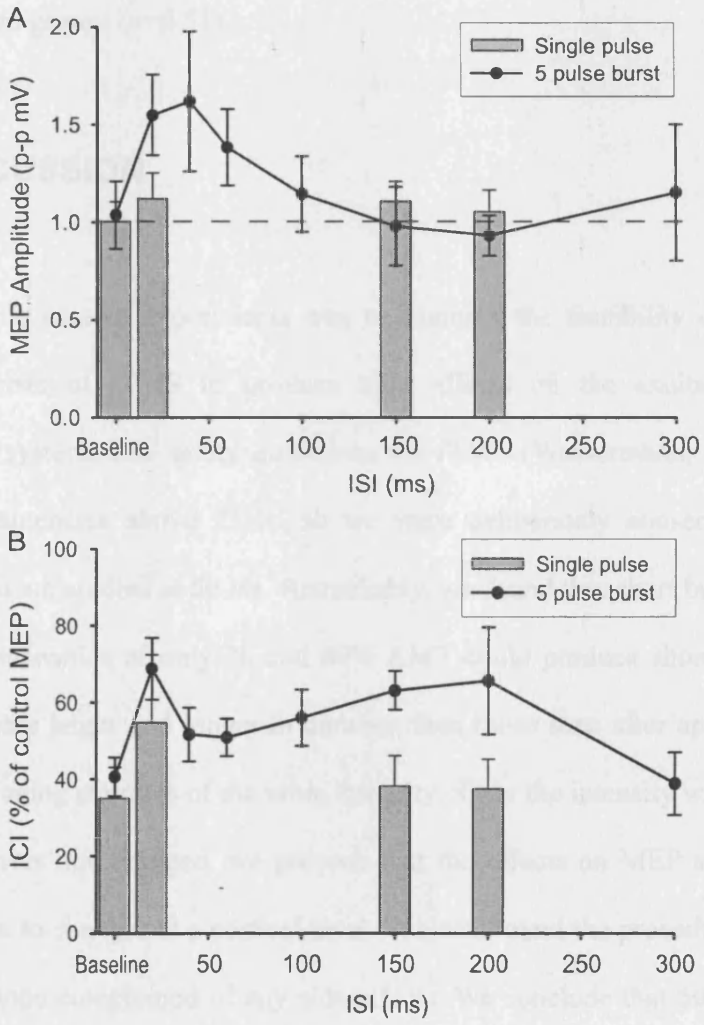

Fig 3.4 ***Comparison of the effect of a single pulse (bars) and a 5 pulse burst at 50Hz (symbols and lines) both at an intensity of 80% AMT.*** *A: The time course of the effect on the MEP amplitude. B: The time course of the effect on SICI presented as percentage of unconditioned MEP. The x axis represents the interval between the single pulse or the last pulse in the conditioning train and the time of the test MEP. This figure represents group data. Error bars refer to the standard error of the measurements.*

The percent SICI (figure 3.4B) was analysed in the same way. Although there was no interaction between TIME and PULSE number, there was a significant main effect of PULSE number ($F(1,12)=7.14$, $p<0.05$), which subsequent paired t tests showed was due to the fact that at 150ms and 200ms, SICI was reduced after the burst but not after a single pulse ($p<0.05$). There was no difference in the control SICI in the two groups ($p=0.51$).

3.4 DISCUSSION

The aim of the present experiments was to examine the feasibility of using high frequency bursts of rTMS to produce after effects on the excitability of the corticospinal system. The safety guidelines for rTMS (Wassermann, 1998) do not extend to frequencies above 25Hz, so we were deliberately conservative in the intensities that we applied at 50 Hz. Remarkably, we found that short bursts of rTMS at 50 Hz at intensities of only 70 and 80% AMT could produce short lasting after effects that were larger and longer in duration than those seen after application of a single conditioning stimulus of the same intensity. Since the intensity was so low and the H-reflex was not changed, we propose that the effects on MEP amplitude and SICI were due to changes at a cortical level. Subjects found the procedure extremely benign, and none complained of any side effects. We conclude that 50 Hz rTMS at low intensity may be a useful form of conditioning for the cerebral cortex in vivo in humans. Future experiments are required to assess the possibility of applying repeated short bursts of high frequency rTMS in a theta burst paradigm.

Because we were interested in the possibility of theta burst applications, in which only a small number of stimuli are given in each burst, we compared the effect of 50 Hz bursts of 5 or 15 pulses. At the intensities we used, both had the same effect on MEP and SICI when tested 20 ms after the end of the burst, and therefore we chose to investigate the detailed time course of the effect following a 5 pulse burst. It should be noted that since this was a feasibility study prior to applying a theta burst paradigm, we did not investigate in detail the mechanisms of the new phenomena that these experiments revealed.

3.4.1 Effect on MEP amplitude

A single conditioning stimulus at 80% AMT had no effect on the amplitude of subsequent test MEPs at intervals of ≥ 20 ms. However, a burst of 5 pulses increased test MEPs at 20 and 40 ms after the end of the burst. As noted above, the low intensity of the stimuli in the burst is unlikely to have produced any descending activity in the corticospinal tract and therefore this increase in excitability is likely to be cortical in origin. The mechanism is unclear, although it is interesting to note that Valls-Sole et al (Valls-Sole et al., 1992) also saw facilitation of test MEPs at about 50 ms after a single, larger, conditioning stimulus. One possibility is that in the present study, the stimuli in the burst were at around the threshold intensity for intracortical facilitation (Ziemann et al., 1996; Orth et al., 2003). If so, then the first stimulus of the burst might have produced a subthreshold period of intracortical facilitation which was still present when the second pulse of the burst was applied 20 ms (i.e. 50 Hz) later. This could continue for the remaining 3 pulses of the burst and result in a period of cortical facilitation resembling that seen after a single larger conditioning stimulus.

3.4.2 Effect on SICI

As with the MEP, a burst of 5 pulses had a stronger effect on SICI than a single pulse of the same intensity. However, the time course of the effect on SICI was longer than that on the MEP, suggesting that (at least at intervals of 150 and 200 ms) that they were mediated by two separate mechanisms.

There have been two previous studies of the effects of a single conditioning stimulus on SICI in the motor cortex, but the intensity of the stimulus and the interval at which SICI was tested were different to those used here. Sanger et al (2001) found that SICI was decreased 100 ms after a suprathreshold stimulus; Bestmann et al (2004) found that a stimulus that alone produced no SICI could increase SICI at an interval of 4 ms or less. The present data appear to show that a single stimulus of 80% AMT had no significant effect on SICI when tested 20-200ms later.

The increase in the effect on SICI of a 5 pulse burst is not unexpected and implies some form of facilitation of the effects of each single pulse when given at a frequency of 50 Hz. However, the interpretation of the effect on SICI is complicated by the fact that the burst also increases the amplitude of MEPs. There are two possible confounding factors. First, the amplitude of the test MEP in the SICI paradigm is increased by rTMS. However, this is usually associated with an small increase in the amount of SICI (Chen et al., 1998) rather than the reduction we saw in the present experiments. In addition we still observed a significant decrease in SICI in three subjects in whom we adjusted the test intensity to maintain the

amplitude of the test MEP following a burst to be 1 mV. The second confounding factor is that previous work has shown that SICI is maximal at particular conditioning intensities (about 80-100% AMT at an SICI interval of 3 ms), and is smaller at intensities above and below that value. The 50 Hz burst, by analogy with its effect on the MEP, could have increased the effectiveness of the conditioning pulse and hence decreased SICI. This seems unlikely to have happened since we found that the 50 Hz burst increased the threshold for producing SICI and reduced the amount of inhibition at all intensities of conditioning stimulus. Finally, it should be noted that the threshold for SICI was expressed relative to AMT, and that control experiments confirmed that this was not affected by rTMS. We therefore conclude that the 50 Hz burst effect on SICI was not secondary to an effect on the excitability of the test or conditioning pulse used to measure SICI.

The 5 pulse burst of 50 Hz reduced SICI for about 200 ms. It is possible that the reduced SICI at long intervals (e.g. 150 and 200 ms) is linked to the observations of Sanger et al (2001) of a similar reduction at the same intervals after a single suprathreshold pulse. Perhaps 5 pulses of low intensity at 50 Hz can summate and recruit this same mechanism. Sanger et al (2001) explained their effect as being due to an interaction between long interval intracortical inhibition (LICI) and SICI. If the 50 Hz burst were recruiting this effect, it might be a useful method of invoking LICI without the preceding MEP that a single large conditioning stimulus evokes.

3.5 CONCLUSIONS

In conclusion, the present results confirm that it is possible to condition the human

motor cortex with short bursts of 50 Hz rTMS using very low intensity pulses. This means that it may be possible in future experiments to apply theta burst conditioning safely to the human cortex to study long-term potentiation at cortical synapses in the same way as it has been so successfully applied in animal experiments.

Chapter 4 Theta burst stimulation

Direct electrical stimulation of central nervous pathways in experimental animals can be used to manipulate synaptic efficiency. Development of transcranial methods of non- invasively stimulating the human brain raised hopes that similar effects could be produced in humans, with the potential for therapeutic application in disease. However, largely due to safety limitations, human subjects often require lengthy conditioning with rTMS, and effects are often weak, variable and not beneficial in the therapeutic setting.

We present studies in humans using novel rTMS paradigms based on theta burst (TBS) patterns. Very short (20 - 190 seconds) conditioning using different TBS patterns at low intensity (80% of active motor threshold) can safely produce controllable, consistent, long-lasting and powerful LTD and LTP-like effects on the motor system in conscious humans at an electrophysiological and behavioural level. This technique has notable advantages over traditional rTMS as an experimental and a potential therapeutic tool.

4.1 INTRODUCTION

Long-lasting changes in the efficacy of synaptic transmission form the basis of neural plasticity (Ridoult-Pedotti and Donoghue, 2003). The ability to control and manipulate this process would be of considerable advantage in the therapy of individuals with particular neurological disorders, as well as being a useful tool to study function in the human brain. In animal preparations, experimental methods

have long been available that can reliably produce opposing changes in the efficacy of synapses in the cerebral cortex. These rely on repeated direct electrical stimulation of neural pathways and can lead to long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission (Larson and Lynch, 1989; Hess and Donoghue, 1996, 1996). One of the most effective patterns of stimulation for producing LTP is the "theta burst" paradigm (Capocchi et al., 1992), which was developed to mimic the normal pattern of neural firing in the hippocampus of rats during exploratory behaviour (Diamond et al., 1988). It consists of very short bursts of high frequency stimulation at 100-200Hz which are repeated at 4 to 7 Hz (the theta range of frequencies in EEG terminology) for a period of around 2s. LTD, in contrast, is often induced by slow frequency (<10 Hz) stimulation (Hess and Donoghue, 1996), although in some systems, by changing the membrane potential, continuous high frequency stimulation may have a similar effect (Randic et al., 1993).

Repetitive transcranial magnetic stimulation (rTMS), which is a non-invasive method of stimulating the brain of conscious human subjects through the intact scalp, has obvious potential for mimicking the effects that have been observed in animal models. Yet despite the striking effects on synaptic transmission that have been achieved in animals, translation to the human brain using rTMS has been relatively disappointing. There are two possible reasons for this: first, even in animal experiments, it has been difficult to demonstrate LTP/LTD in the cortex of awake and freely moving animals without the use of extended or repeated sessions of stimulation (Trepel and Racine, 1998; Froc et al., 2000). Second, concerns over safety have limited most studies on humans to the use of low frequencies of

stimulation (Wassermann, 1998). This type of stimulation can certainly produce effects on neural systems in humans that outlast the period of stimulation, and which have some characteristics that are similar to those seen in animal studies (Maeda et al., 2000; Touge et al., 2001; Siebner and Rothwell, 2003). However, conditioning with low frequencies of stimulation is slow, and in human studies the ratio of the time taken to apply the stimulation to the duration of the after-effect is only about 1:1 (Siebner and Rothwell, 2003), which is clearly not practical for a therapeutic application. In addition, the effect observed after rTMS in humans is subject to notable inter-individual variability (Maeda et al., 2000; Sommer et al., 2002), and behavioural effects have been elusive (Chen et al., 1997; Muellbacher et al., 2000) without the use of complex experimental paradigms (Schlaghecken et al., 2003). Current methods of rTMS therefore fall some distance short of providing an effective, reliable and controllable tool with which to produce plastic changes in the human brain.

In view of these difficulties, we have explored whether high frequency bursts of rTMS, albeit delivered at very low intensities to maintain safety, might be a more effective than single pulses in inducing changes in the excitability of cortical circuits in conscious humans. In previous chapter, we found that even single bursts of 5 stimuli can have short-lasting after effects superior to those achieved by a single pulse (Huang and Rothwell, 2004). Here we have developed this technique to deliver trains of high frequency bursts in different patterns to produce controllable effects on motor cortical excitability and behaviour which are quick to produce, powerful and reliable.

4.2 METHODS AND SUBJECTS

4.2.1 Subjects

All subjects for these experiments were healthy volunteers between the ages of 23 and 52 (mean age: 33.6±7.8 years). All subjects gave their informed consent for the experiments, and the project protocol was approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery.

4.2.2 Stimulation and recording

For all experiments, subjects were seated in a comfortable chair. EMGs were recorded using Ag-AgCl electrodes from the right first dorsal interosseous (the dominant hand in all subjects). EMG activity was recorded with a gain of 1000 and 5000 and filtered with a band-pass filter (3 Hz to 2k Hz).

Magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK). Single and paired pulses were delivered by Magstim 200 machines, and rTMS was delivered using a Magstim rapid stimulator connected to four booster modules. Stimulation was delivered over the motor hand area with the coil placed tangentially to the scalp with the handle pointing posteriorly. The motor hand area was defined as the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral first dorsal interosseous (FDI) when the subject was relaxed (the "motor hot-spot"). The stimulation intensity was defined in relation to the active motor

threshold (AMT) of the subject. The AMT was defined for each Magstim machine separately as the minimum intensity of single pulse stimulation required to produce an MEP of greater than 200μV on more than five out of ten trials from the contralateral FDI while the subject was maintaining a voluntary contraction of about 20% of maximum in the FDI. Visual feedback was provided to the subject to help maintain a constant muscle contraction of the correct force.

4.2.3 Experiments

4.2.3.1 The TBS paradigms

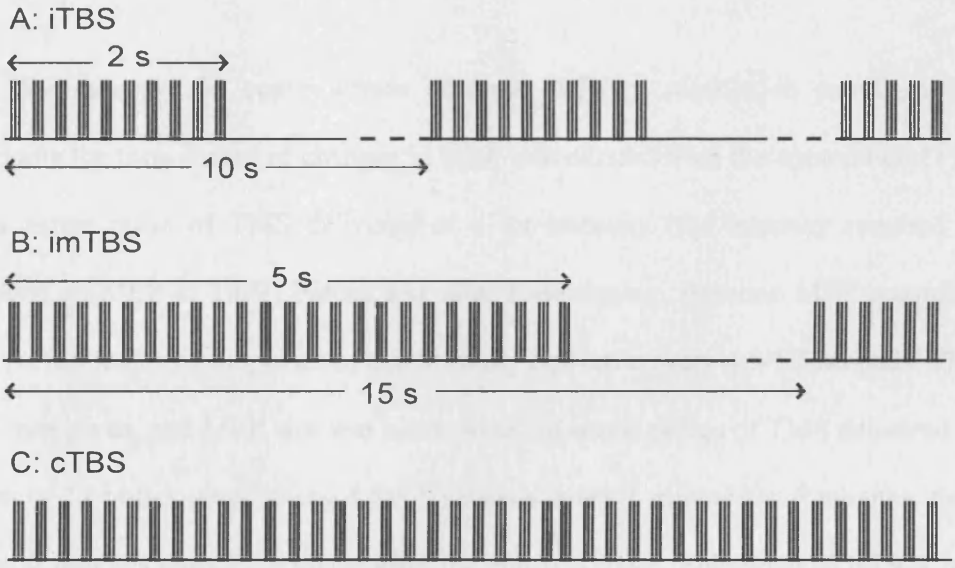

Fig 4.1 ***A graphical illustration of the three stimulation paradigms used.*** *Each paradigm uses a theta burst stimulation pattern (TBS) in which 3 pulses of stimulation are given at 50Hz, repeated every 200ms. In the intermittent theta burst stimulation pattern (iTBS), a 2s train of TBS is repeated every 10s for a total of 190s (600 pulses). In the intermediate theta burst stimulation paradigm (imTBS) a 5s train of TBS is repeated every 15s for a total of 20 times (600 pulses). In the continuous theta burst stimulation paradigm (cTBS) a 40s train of uninterrupted TBS is given (600 pulses).*

The basic TBS pattern that we developed for these experiments was a burst containing 3 pulses of 50Hz magnetic stimulation at 80% AMT given every 200ms (i.e. at 5Hz). We used this basic pattern four different stimulation paradigms. 1) Intermittent theta burst stimulation (iTBS): the basic pattern given in a short train lasting 2 seconds (i.e. 10 bursts), repeated every 10 seconds for 20 cycles (a total of 600 pulses). 2) Intermediate theta burst stimulation (imTBS): the basic pattern given in a longer train lasting 5 seconds (i.e. 25 bursts), repeated every 15 seconds for 8 cycles (a total of 600 pulses). 3) Continuous theta burst stimulation (cTBS): the basic pattern given in a continuous train lasting 40 seconds (i.e. 200 bursts; total of 600 pulses) or 20 seconds (cTBS300) (i.e. 100 bursts; total 300 pulses). A graphical illustration of these paradigms is shown in Fig 4.1 A,B,C.

We assessed the consequences of these different stimulation paradigms by assessing the time course of changes in MEP size elicited from the contralateral FDI by a single pulse of TMS delivered at a set intensity (the intensity required to produce an MEP of 1mV) before and after conditioning. Baseline MEP recording was performed using 30 pulses of this intensity delivered every 4.5-5.5 seconds. TBS was then given, and MEP size was assessed using single pulses of TMS delivered in trains of 12 pulses given every 4.5-5.5 seconds every 1 minute for 6 minutes, then every 2 minutes until 21 minutes after the end of iTBS. Following cTBS600, we performed the same assessment of MEP size, but extended the assessment, testing MEP amplitude every 4 minutes until 61 minutes after the end of conditioning. Nine subjects (6 men, 3 women; mean age, 30±6 years) took part in these experiments.

4.2.3.2 The effect of 15Hz rTMS

As a comparison with the TBS paradigms, one experiment was performed using

15Hz rTMS on seven out of the nine subjects used in the TBS paradigm experiments (5 men, 2 women; average age 37±9 years). We delivered 15Hz rTMS continuously for 20 seconds at an intensity of 80% of AMT over the motor hand area in relaxed subjects. We assessed the response to this stimulation by assessing MEP size before and after stimulation according to the paradigm described above.

4.2.3.3 The effect of TBS on short intracortical inhibition and facilitation

We adjusted the intensity of the test stimuli while assessing SICI and ICF after TBS to maintain the amplitude of test MEPs at approximately 1 mV.

iTBS

We assessed short interval intracortical inhibition (SICI) and facilitation (ICF) on seven subjects (5 men, 2 women; mean age, 30±7 years) in the motor hand area before and after iTBS using the double-pulse method described by Kujirai et al. We assessed SICI at an interstimulus interval (ISI) of 2ms using a conditioning intensity of 80% AMT, and ICF at an ISI of 10ms with a conditioning intensity of 90% AMT. Two blocks of baseline SICI and ICF were recorded. After iTBS, SICI and ICF were recorded every 4 minutes until 20 minutes following conditioning.

cTBS

We assessed SICI and ICF on seven subjects (4 men, 3 women; mean age, 27±3 years) in the motor hand area before and after cTBS300. We assessed SICI at ISIs of 2 and 3ms using a conditioning intensity of 80% AMT, and ICF at ISIs of 10 and 15 ms with a conditioning intensity of 90% AMT. Two blocks of baseline SICI and ICF were recorded. After cTBS, SICI was recorded every five minutes until 25 minutes

after conditioning, while ICF was only recorded at 10 minutes, where we found the peak effect of cTBS to occur, and then at 30 minutes after cTBS.

To assess the effect of cTBS on spinal motor excitability, we also tested the H-reflex and MEP in the contralateral flexor carpi radialis (FCR) muscle before and after cTBS on seven subjects (6 men, 1 woman; mean age 31±7 years). One block mixing 12 trials of H-reflex and 12 trials of MEP was recorded prior to conditioning, and another block was recorded at 10 min after cTBS.

4.2.3.4 The effect of cTBS on motor threshold and LICI

Rest and active motor threshold and LICI at an ISI of 100 ms were measured before and at 10 min after the end of cTBS300 as described in chapter 4. The intensity of the conditioning (first) stimulus was adjusted in each subject prior to cTBS so that it produced about 50% of inhibition of the test (second) MEP. The test stimulus was set at an intensity that would evoke an EMG response of around 1 mV peak-to-peak amplitude, when given alone. One block of 10 trials was given with intertrial intervals ranging randomly between 4.5-5.5 sec. RMT and AMT were measured before and at 8 minutes after the end of cTBS. Then 2 blocks of LICI were assessed. The same conditioning intensity as used in the baseline assessment was used in one of them. In the other I used an intensity adjusted for any change in RMT. The two blocks were given in a random sequence.

4.2.3.5 Reaction Time

In a separate experiment, we assessed reaction time before and after cTBS. Nine

subjects (6 men, 3 women; mean age, 33±7 years) were recruited for this experiment. Subjects were seated in a comfortable chair, with each index finger placed on a button. Ag/Ag-Cl electrodes were attached to ulnar side of both hands. An electrical stimulus was delivered through these electrodes at an intensity of 3 times sensory threshold. Subjects were instructed that when they felt a stimulus on the right or the left hand, that they were to press the button under the corresponding finger as quickly as possible. In addition, subjects were asked to press the button with a particular force (approximately 2.5 N). Visual feedback as to the accuracy of the force with which they pressed the button was given on a screen in front of the subject.

Two blocks of reaction time testing were performed, with 40 stimuli to each hand given at random intervals, ranging from 1.5 to 2.5 seconds, and in a random pattern. cTBS was then given over the left motor hand area, and then two similar blocks of reaction time testing were performed starting at 10 minutes after cTBS. Reaction time was assessed in a similar fashion once more at 30 minutes after cTBS.

4.2.3.6 TBS-like paradigm: slow theta burst stimulation

A TBS-like paradigm was also investigated in the same 9 subjects as used in TBS experiments. In this paradigm, which I have called slow theta burst stimulation (sTBS), five pulses of 50Hz stimulation were given every 1 s in a continuous train lasting 60 seconds (a total of 300 pulses). Two intensities, 80% and 90% AMT, were tested in this experiment. I assessed the consequences of these different stimulation paradigms by assessing the time course of changes in MEP size elicited from the contralateral FDI by a single pulse of TMS delivered at a set intensity (the intensity

required to produce an MEP of 1mV) before and after conditioning. Baseline MEP recording was performed using 30 pulses of this intensity delivered every 4.5-5.5 seconds. TBS was then given, and MEP size was assessed using single pulses of TMS delivered in trains of 12 pulses given every 4.5-5.5 seconds every 5 minutes until 35 minutes after the end of the sTBS paradigm.

4.2.4 Data analysis

Data were analysed using SPSS for Windows version 11.0. Repeated measures ANOVA was used to compare variables before and after TBS, and paired t-tests were used to compare the effect of TBS on H-reflexes and MEPs recorded from FCR and the effect of a single pulse. Statistics for the data in Figs 4.2A and for the effect of sTBS at different intensities were performed on absolute amplitude values rather than the normalised amplitudes that are plotted in the graphs, whereas the statistics for comparing the effect of iTBS, imTBS and cTBS600 and comparing the effect of cTBS 300 and sTBS were performed on normalised data. The comparison of data between MEP and H reflex was performed on log transformed values in order to normalise the distribution of the amplitude data.

4.3 RESULTS

Side effects were not observed or reported in any subjects during or following any of the experiments.

4.3.1 The effect of different theta burst stimulation (TBS) paradigms on MEP amplitude

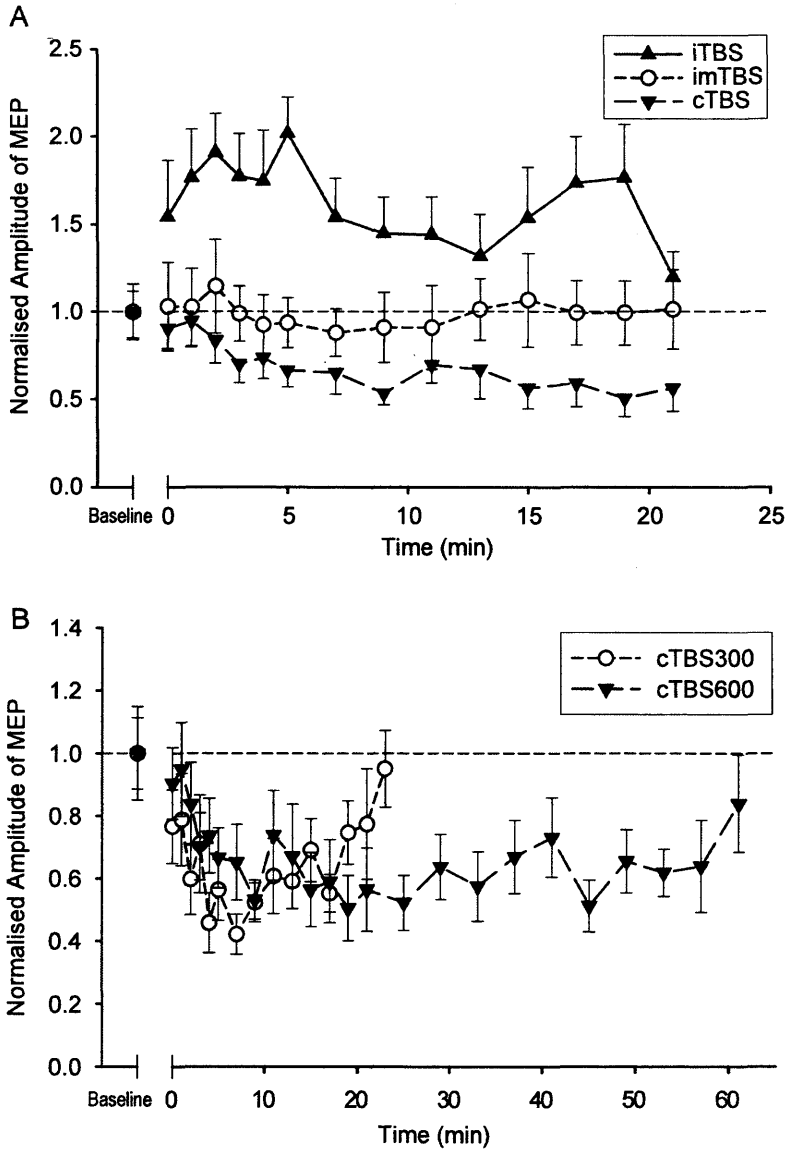

Fig 4.2 ***The time course of changes in MEP amplitude following different conditionings.*** *Fig 4.2A shows the time course of changes following conditioning with iTBS (▲), cTBS (▼), or imTBS (○). There was a significant effect of pattern of stimulation on change in MEP size following stimulation ($F(2,16)=20.32$, $p<0.001$), with significant post hoc differences between each pattern of stimulation. There was a significant facilitation of MEP size following iTBS lasting for about 15 min, and a significant reduction of MEP size following cTBS lasting for nearly 60 min. imTBS produced no significant changes in MEP size. Fig 4.2B compares the effects of cTBS given for 20s (300 pulses; cTBS300 (○)) with the same paradigm given for 40s (600 pulses; cTBS600(▼)). There was a significant effect of duration of cTBS conditioning on the time course of the effect (significant TIME x DURATION interaction ($F(14,112)=2.24$, $p<0.05$)) with the effect of cTBS300 lasting about 20 minutes compared to the effect of cTBS600 which lasted about 60 minutes.*

We evaluated the effect of intermittent theta burst stimulation (iTBS), intermediate theta burst stimulation (imTBS) and continuous theta burst stimulation (cTBS) on MEP amplitude. Figure 4.2A shows the amplitude of MEPs elicited before and after applying iTBS, imTBS or cTBS to the motor cortex until 21 minutes after the TBS stimulation. A two way ANOVA comparing normalized amplitude of MEPs following three different paradigms of TBS revealed a significant effect of PATTERN (i.e. iTBS, imTBS, or cTBS) ($F(2,16) = 20.32$, $p < 0.001$). Post hoc analysis showed a significant difference between each pattern of TBS (iTBS vs. imTBS: $p<0.01$; imTBS vs. cTBS: $p<0.05$; iTBS vs. cTBS: $p<0.001$). When the stimuli were applied in the iTBS pattern, MEPs were facilitated for about 19 minutes ($F(14,112)=1.99$, $p<0.05$). In contrast, the same number of stimuli applied in a cTBS pattern suppressed MEPs for about 60 minutes ($F(24,192)=3.33$, $p<0.001$). The same number of stimuli in an imTBS pattern had no effect on MEPs ($F(14,112)=0.41$).

The amplitude of MEPs elicited before and after applying cTBS to the motor cortex for 20 seconds (cTBS300) or 40 seconds are shown in Fig 4.2B. A two factor ANOVA with TIME and DURATION (i.e. 20 seconds or 40 seconds) as main factors revealed a significant TIME x DURATION interaction ($F(14,112)=2.24$, $p<0.05$). cTBS given for 20 seconds suppressed MEPs for around 20 minutes, while cTBS given for 40 seconds suppressed them for up to 60 minutes.

4.3.2 Comparison of TBS paradigms with traditional 15Hz rTMS

We compared the effect of cTBS300 with continuous stimulation at 15 Hz for 20 seconds, in which 300 pulses were given evenly in the same duration of cTBS300 using a two way ANOVA (Fig 4.3). This revealed a significant interaction between

TIME and PATTERN ($F(14,84)=2.55$, $p<0.005$), with post hoc analysis demonstrating that continuous stimulation at 15 Hz for 20 seconds had no effect on MEP amplitude ($F(14,84)=1.63$).

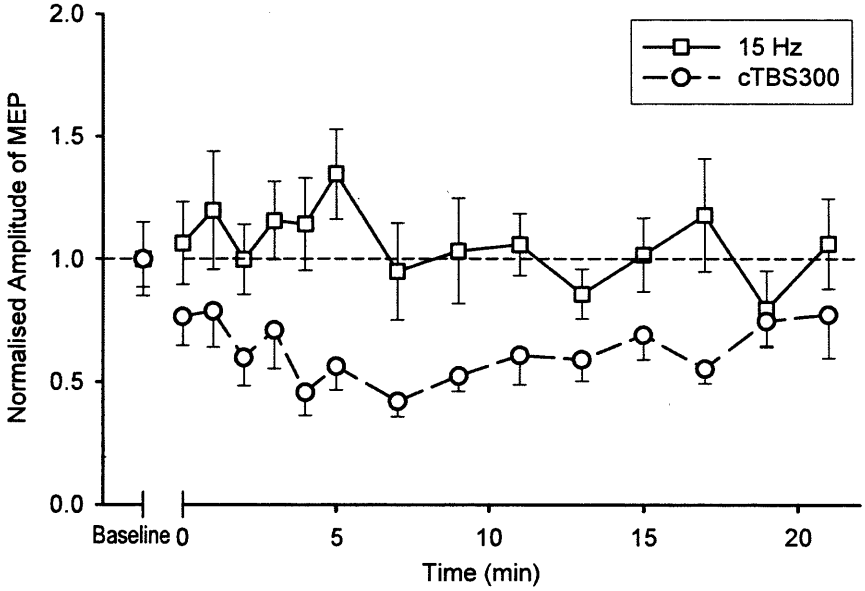

Fig 4.3 ***The comparison of the effect of continuous 15Hz stimulation for 20s (□) (300 pulses) with cTBS given for 20s (○) (300 pulses).*** *Only the cTBS paradigm had any effect on MEP size following stimulation, and there was a significant interaction between TIME and PATTERN ($F(14,84)=2.55$, $p<0.005$).*

4.3.3 Effects of TBS on short intracortical inhibition, intracortical facilitation and spinal motor function.

Given the very low intensity of the individual pulses (80% AMT), it is highly unlikely that TBS produced any activity in descending corticospinal fibres, and therefore that there were any direct effects of TBS on the excitability of circuits in the spinal cord. However, as a further test of this we compared the effect of cTBS300 on MEPs evoked in forearm flexor muscles with that on the spinal H-reflex evoked

in the same muscles. This experiment found that MEP size was suppressed in these muscles following cTBS 300 (t=2.88, p<0.05), but that H reflex size was not significantly different (t=-1.55, ns). Due to large inter-individual variability of H reflex and MEP size elicited from FCR, we took the logarithm of these data. After this calculation, MEP size and H reflex size following cTBS 300 showed a significant interaction ($F(1,7)$=6.05, p<0.05).

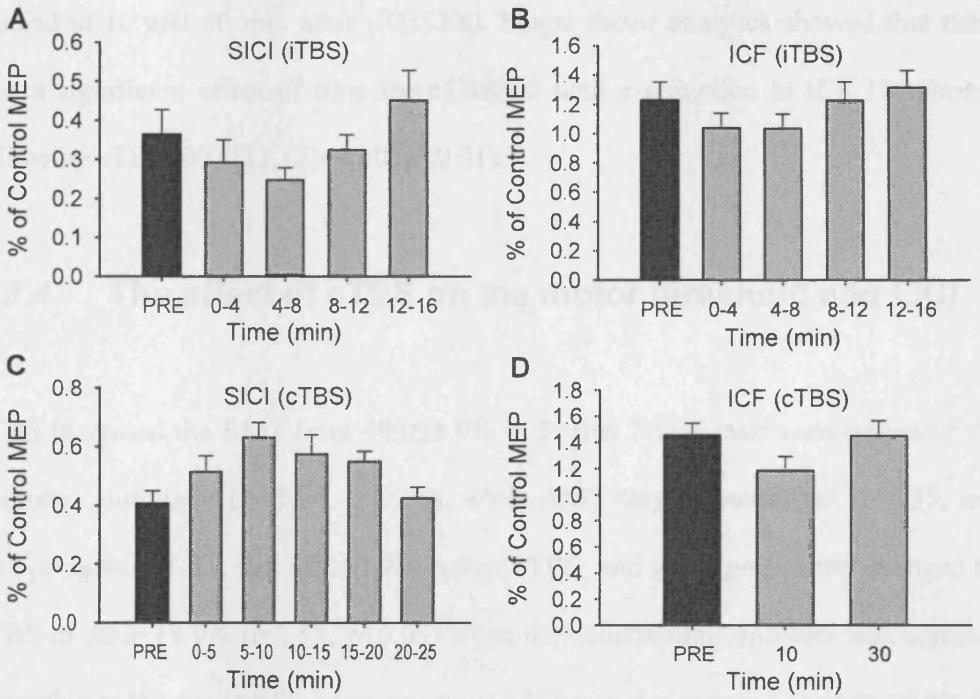

Fig 4.4 ***The effect of iTBS and cTBS on short intracortical inhibition (SICI) and facilitation (ICF).*** *(a) SICI was significantly increased following iTBS ($f(4,24)$=5.01, p<0.005), but (b) was reduced following cTBS ($f(5,30)$=3.75, p<0.01). (c) ICF was not significantly altered following iTBS, but (d) was significantly reduced at 10 min following cTBS ($f(2,12)$=7.40, p<0.01).*

As further evidence that TBS has an effect on the excitability of intrinsic cortical circuits, we measured short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) before and after iTBS and cTBS300 using a standard paired pulse paradigm.

Fig 4.4A shows the data for SICI following iTBS. SICI was significantly increased following iTBS (One factor ANOVA on the time course: $F(4,24)=5.01$, $p<0.005$). Fig 4.4B shows the data for ICF following iTBS. No significant effect was observed on ICF following the conditioning.

Fig 4.4C shows that cTBS reduced the amount of SICI for the 20min following conditioning ($F(5,30)=3.75$, $p<0.01$). Fig 4.4D shows the data for ICF, which was studied at 10 and 30 min after cTBS300. Single factor analyses showed that there was a significant effect of time for cTBS300 with a reduction in ICF 10 minutes following cTBS300 ($F(2,12)=7.40$, $p<0.01$).

4.3.4 The effect of cTBS on the motor threshold and LICI

cTBS increased the RMT from 49.0±8.9% to 51.0±9.7% of maximum output of the magnetic stimulator (t=-3.24, p<0.05), while AMT stayed unchanged (t=0.55, ns). The percentage LICI was 58.1±7.7% before cTBS, and was significantly changed by cTBS to 38.3±19.9% (t=2.68, p<0.05) when the conditioning intensity was adjusted according to the new RMT. The percentage LICI was also reduced to 46.8±20.8% by cTBS when the conditioning intensity was not adjusted, although it was not statistically significant (t=1.429).

4.3.5 Behavioural effects of TBS

cTBS300 produced clear changes in simple reaction times. In this experiment, cTBS300 was applied to the left motor cortex and reaction times measured in the right (conditioned) and left (unconditioned) hands (Fig 4.5). A two factor ANOVA

revealed a significant interaction between time (before and after cTBS300) and hand ($F(2,16)=4.30$, $p<0.05$.) indicating that cTBS300 had a different effect on the reaction times of the two hands. One factor analyses showed that there was a significant effect of time in both hands (conditioned hand: $F(2,16)=12.77$, $p<0.001$; unconditioned hand: $F(2,16)=7.82$, $p<0.005$) However, in the unconditioned hand this was due to a decrease in reaction times 30 min after cTBS300, whereas in the conditioned hand it was due to an increase in reaction time 10 min after cTBS300. The accuracy of the force with which subjects pressed the button was not changed in either hand following conditioning (conditioned hand: $F(2,16)=0.18$, ns; unconditioned hand: $F(2,16)=1.14$, ns).

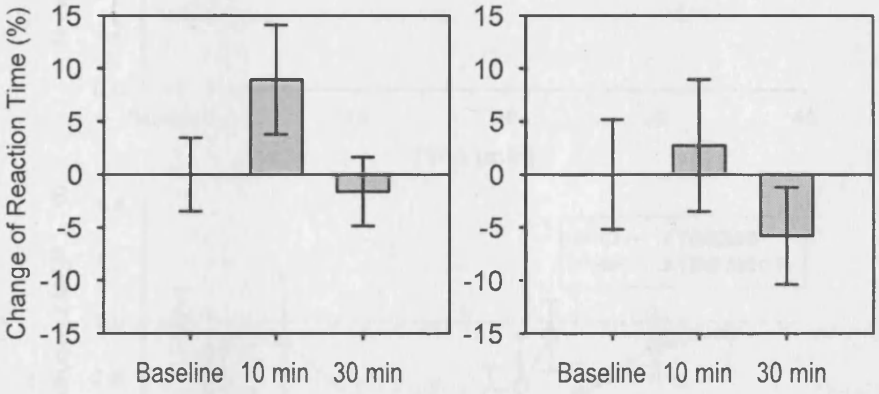

Fig 4.5 ***The changes in simple reaction time following cTBS.*** *There was a significant lengthening of reaction time in the conditioned hand 10 min after cTBS ($F(2,16)=4.30$, $p<0.05$; Fig4.5A), and a significant shortening of reaction time in the unconditioned hand 30 min after cTBS ($F(2,16)=7.82$, $p<0.005$; Fig 4.5B).*

4.3.6 The effect of sTBS on MEP amplitude

MEPs were suppressed by sTBS at both 80% ($F(8,64)=2.09$, $p<0.05$) and 90% ($F(8,64)=2.70$, $p<0.05$) AMT (Fig 4.6A). Post hoc testing revealed significant suppression at 10, 15 and 20 min after sTBS at 80% AMT, and at 5, 10, 15 and 20 min after sTBS at 90% AMT. A two factor ANOVA showed no interaction between

conditioning INTENSITY (80% and 90%) and TIME ($F(8,64)=0.52$, *ns*) nor any main effect of INTENSITY ($F(1,8)=0.01$, *ns*). We also compared the effect of sTBS and cTBS300 (Fig 4.6B) and found a significant interaction between TIME and PATTERN ($F(5,30)=2.60$, $p<0.05$).

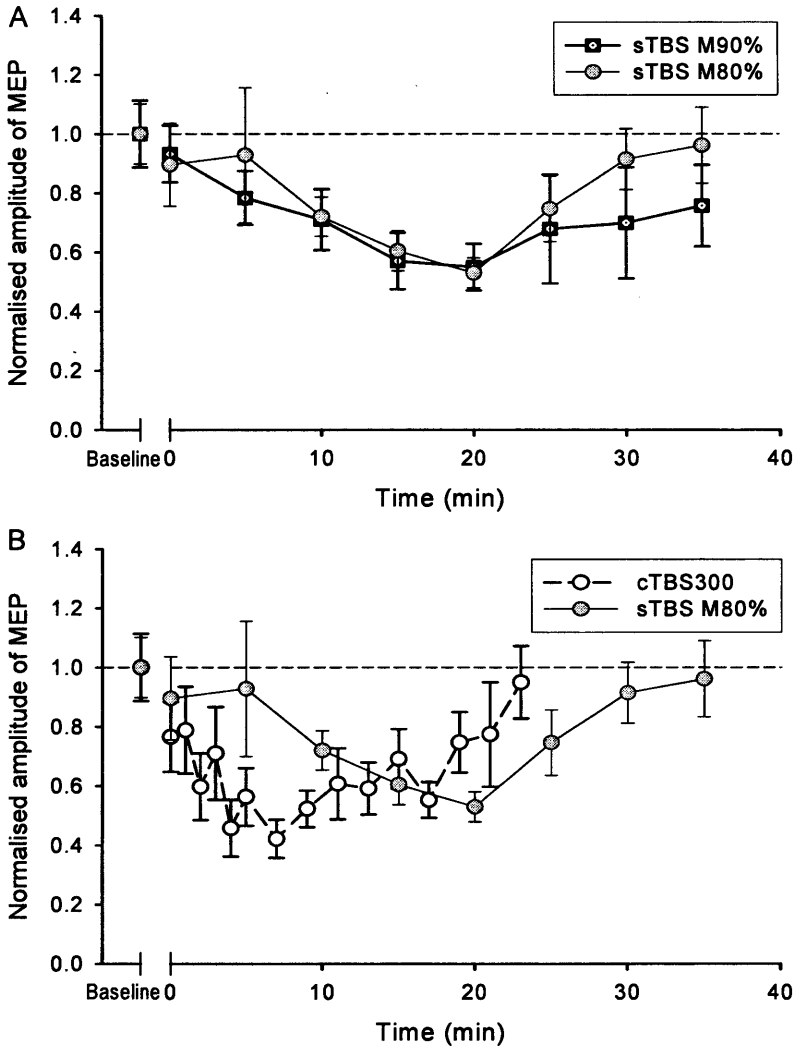

Fig 4.6 ***The time course of changes in MEP amplitude following sTBS.*** *(A) There was a significant reduction of MEP size following sTBS at 80% or 90% AMT lasting for about 20 min ($F(8,64)=2.09$, $p<0.05$; $F(8,64)=2.70$, $p<0.05$, respectively). (B) The effects of sTBS at 80% AMT and cTBS with the same number of pulses at the same intensity were compared. There was significant interaction between TIME and PATTERN ($F(5,30)=2.60$, $p<0.05$).*

4.4 DISCUSSION

4.4.1 The effect of TBS

We have demonstrated that stimulation over the motor hand area in healthy subjects using frequencies of rTMS based on theta burst patterns can produce rapid changes in the function of the motor system that outlast the period of stimulation by over 20 minutes. These long-lasting, consistent and significant effects were produced despite very short periods of conditioning (20 – 190 seconds) and very low stimulus intensities (80% of AMT). This is in contrast to many previous paradigms of rTMS in humans, which have required much longer periods of conditioning at higher stimulus intensities to have an effect of a similar duration (Touge et al., 2001).

The data show that the effects on the motor system depend on the pattern of stimulation. Thus, continuous TBS reduces corticospinal excitability, as indicated by the decline in MEP amplitudes, whereas the same number of pulses given continuously over the same period of time (20 seconds of 15Hz rTMS) has no effect. TBS applied in an intermittent pattern (iTBS) can increase corticospinal excitability, indicated by the increase in MEP amplitudes after this type of conditioning. We have also shown that cTBS300 can produce clear behavioural effects that last for at least 10 min after conditioning in a simple reaction time task. Taken together, these studies demonstrate that the application of TBS patterns of rTMS to the human brain for very short periods can have substantial effects on cortical circuitry.

Two lines of evidence suggest that TBS is having an effect on circuitry intrinsic

to the motor cortex. First, MEPs evoked in the forearm flexor muscles by a standard pulse of TMS were smaller after cTBS300, whereas spinal H-reflexes in the same muscle were unaffected. The simplest explanation for this is that spinal motoneurones and the synaptic input from the H-reflex are unaffected by cTBS, and that changes in MEP are due to changes in the excitability of circuits in the cortex. Since MEPs evoked in hand and forearm muscles by TMS pulses are produced by trans-synaptic excitation of corticospinal projection neurones (Di Lazzaro et al., 1998), this would imply either an increase in the effectiveness of that synaptic input or an increase in the baseline excitability of the corticospinal neurones that receive it. A lack of effect of TBS on spinal circuits would also be consistent with the very low intensity (80% AMT) of the pulses we employed for TBS: well below the threshold for evoking any direct corticospinal effects. However, it is possible that the population of spinal motoneurones tested by the H-reflex is different from that recruited by the MEP, so that a small spinal component to the MEP change cannot be entirely ruled out.

The second reason for suspecting that the TBS effect is cortical is the change that it produces in the results of paired pulse testing. The paradigms that test SICI and ICF in humans are thought to monitor excitability in local intracortical circuits, some of which involve GABAa-ergic connections (Kujirai et al., 1993; Ziemann et al., 1996). This makes it highly likely that a change in SICI and ICF is due to an effect on the excitability of connections in these circuits. In line with the MEP data, effects of TBS on SICI and ICF depend on the pattern of stimulation used (intermediate vs. continuous). Thus both SICI and ICF are suppressed following cTBS300 relaxed, whereas after iTBS, SICI is increased, and ICF is unchanged. However, not like SICI or ICF, the LICI was enhanced by cTBS. This may suggest

that cTBS produces the LTD-like effect through the same or a common pathway of LICI.

Our finding of a significant slowing of reaction time following just 20 seconds of cTBS is notable, as a clear behavioural effect on such a simple task has been difficult to produce previously with other kinds of rTMS (Chen et al., 1997; Muellbacher et al., 2000). It seems likely that the significant shortening of reaction time that was observed in the unconditioned hand 30 minutes after conditioning is a learning effect. A similar effect was not observed in the conditioned hand, perhaps indicating that conditioning with cTBS not only affected reaction time, but also motor learning. It is possible also that the hypofunction of the left hemisphere caused by TBS produced a reciprocal hyperfunction of the right hemisphere, facilitating motor responses in the unconditioned hand via a similar mechanism to that observed in human subjects following unilateral stroke (Delvaux et al., 2003). However, further experiments are needed to address these possibilities directly.

It has been suggested that a longer conditioning stimulation can cause a longer and larger effect on brain plasticity (Maeda et al., 2000; Touge et al., 2001). In our experiments, the LTD-like effect caused by cTBS was extended from 20 minutes to 60 minutes by prolonging the duration of the conditioning from 20 seconds to 40 seconds. However, the maximum effect of suppression of MEPs after cTBS for 40 seconds is not larger than the effect of cTBS300. It is possible that the maximum suppression effect is reached after 20 seconds of conditioning, and that all further conditioning is able to produce is a lengthening of the effect.

4.4.2 The influence of frequency and intensity

It is widely accepted that regular rTMS at a frequency not higher than 1 Hz produces an inhibitory effect (Chen et al., 1997; Touge et al., 2001), whereas rTMS at 5Hz or higher produces a facilitatory effect (Berardelli et al., 1998; Maeda et al., 2000). However, the present experiments show that sTBS, in which a burst was given every 1 s (i.e. 1Hz), and cTBS, in which a burst was given every 200 ms (i.e. 5Hz), produce similar inhibitory effects. For a given intensity, cTBS (which actually had the higher basic frequency of 5 Hz compared with 1 Hz) produced stronger and more consistent inhibition than sTBS. The effect of sTBS built up slower and only significantly suppressed MEPs at 10-20 min after the end of the conditioning. We also tried a train containing a 3-pulse 50 Hz burst given every 100 ms (i.e. 10 Hz) continuously for 10 seconds in one subject. It showed very similar suppression to cTBS300. Therefore, unlike with single pulse stimulation, the interval between short high frequency bursts may not be important for determining the direction of the plasticity, although it may affect the size of the effect.

Stimulus intensity has also been suggested to be crucial for determining the direction of the change in synaptic efficiency. Modugno et al. (2001) proposed that the threshold for evoking inhibition is lower than that for facilitation and that suppression produced by a train of rTMS could be converted into facilitation if the intensity of rTMS was increased. However, in the present experiments, when the intensity of sTBS was increased from 80% to 90% AMT, suppression was not converted to facilitation. In fact the suppression was enhanced and built up earlier, although the difference was not statistically significant. However, we did not raise the intensity further due to the safety concerns. It is possible that the intensity needs

to be increased to a suprathreshold level to convert the inhibitory effect to facilitation.

4.5 CONCLUSIONS

In conclusion, we have developed novel methods of delivering rTMS based on patterns of theta burst stimulation. We have found these stimulation paradigms to be safe in normal subjects, and capable of producing consistent, rapid and controllable electrophysiological and behavioural changes in the function of the human motor system that outlast the period of stimulation by over an hour. In particular we have found that the pattern of delivery of TBS (continuous versus intermittent) is crucial in determining the direction of change in synaptic efficiency. These initial findings have implications for both the use of rTMS in the study of human neural physiology, and in the use of rTMS in the therapeutic manipulation of brain plasticity.

Chapter 5 The mechanism of theta burst stimulation

In chapter 4, I presented studies in humans using novel rTMS paradigms based on theta burst (TBS) patterns. Very short (20 - 190 seconds) conditioning using different TBS patterns at low intensity (80% of active motor threshold) can safely produce controllable, consistent, long-lasting and powerful LTD and LTP-like effects on the motor system in conscious humans at an electrophysiological and behavioural level. In particular we have found that the pattern of delivery of TBS (continuous versus intermittent) is crucial in determining the direction of change in synaptic efficiency. However, these studies do not reveal the underlying mechanisms of the observed effects. There is no previous data regarding the crucial role of pattern of stimulation in determining the direction of plasticity-induction even in animal studies. Although people use TBS for LTP induction and a continuous train of TBS-like stimulation lasting 180-190 seconds to induce LTD in brain slices (Heusler et al., 2000) or living animals (Takita et al., 1999), they have never been compared in the same preparation.

In this chapter, the mechanism of TBS will be presented. I shall start it with a triple pulse experiment that can help us understand more about the longer-lasting decrease in SICI after a burst. Then the after-effect of a short train of bursts (10 or 25 bursts) will be described. These results together with the findings in last chapter can provide the clue about how facilitatory and inhibitory effects cascade during TBS. At last, a simple mathematical model will be developed to explain the possible mechanism of repetitive stimulation on the synaptic efficiency.

5.1 INTRODUCTION

Repeated electrical stimulation of neural circuits in the brains of animal preparations can alter the efficiency of synaptic transmission and lead it to long-term potentiation (LTP) or long-term depression (LTD), which are very closely linked to the functional cortical reorganisation (Hess and Donoghue, 1994). Transcranial magnetic stimulation (TMS), a technique for non-invasive stimulation of the human brain, led the expectation to produce the similar effect in conscious human beings (Muellbacher et al., 2000; Siebner and Rothwell, 2003). Reorganisation occurs in the human brain in response to injury or disease (Kaas et al., 1983; Flor et al., 1995; Buchkremer-Ratzmann et al., 1996; Nelles et al., 1999; Huse et al., 2001; Karl et al., 2001), and it would be of considerable benefit to have an effective tool with which to enhance these changes where they are beneficial (e.g. in the recovery of the motor system after stroke) or to suppress them where they are maladaptive (e.g. in the phantom limb syndrome). Repetitive transcranial magnetic stimulation (rTMS) has potential as such a tool to have therapeutic effects on neurological (Siebner et al., 1999; Shimamoto et al., 2001; Gilio et al., 2002; Huang et al., 2004) and psychological (Speer et al., 2000; Daskalakis et al., 2002; Martin et al., 2002) diseases.

Although many cellular and molecular mechanisms have been investigated to elucidate the LTP/LTD effects (Larson and Lynch, 1988; Hirsch and Crepel, 1991, 1992; Randic et al., 1993; Aroniadou and Keller, 1995; Barr et al., 1995; Kaila et al., 1997; Vickery et al., 1997; Otani et al., 1998; Otani and Connor, 1998; Speer et al., 2000; Caria et al., 2001; Patenaude et al., 2003; Yasuda et al., 2003), the protocols of

repetitive stimulation, electrical or magnetic, for plasticity-induction have rarely been discussed. The most commonly accepted theory, which people strongly propose, is that the frequency decides the direction of the plasticity which is induced (Valls-Sole et al., 1992; Valzania et al., 1994) . Low frequency stimulation (LFS) is usually used to produce LTD effect, whereas high frequency stimulation (HFS) can produce an LTP effect. Among many kinds of HFS, repetitive high frequency bursts in a theta burst (TB) pattern is the most common paradigm used to produce LTP in animal preparations.

This "rule of frequency" has worked very well and been followed and used to study the mechanism of LTD or LTP for decades. The rule itself, nevertheless, has never been carefully explored, although it is believed that the pattern of Ca^{2+} influx induced by the stimulation at different frequencies determines the direction of the change in synaptic efficiency. Unlike some other experiments reversing the direction of the plasticity effect of a paradigm by changing the postsynaptic membrane potential (Randic et al., 1993) or stimulation intensity, I have demonstrated that applying theta burst pattern rTMS in the human motor cortex can very quickly produce LTP- or LTD-like effect by using bursts at same frequency and intensity without experimentally changing the membrane potential. The data suggest that when TMS is applied in the "theta burst" pattern it leads to a mixture of excitatory and inhibitory effects that have different efficacy and time course. The proportion of each effect can be controlled by adjusting the timing of the theta bursts so that it is possible to produce powerful and reliable effects on motor cortical excitability and behaviour.

5.2 METHODS AND SUBJECTS

5.2.1 Subjects

All subjects for these experiments were healthy volunteers between the ages of 23 and 38 (mean age: 30 ± 5 years). All subjects gave their informed consent for the experiments, and the project protocol was approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery.

5.2.2 Stimulation and recording

For all experiments, subjects were seated in a comfortable chair. EMGs were recorded using Ag-AgCl electrodes from the first dorsal interosseous (FDI) in the dominant hand. EMG activity was recorded with a gain of 1000 and 5000 and filtered with a band-pass filter (3 Hz to 2k Hz) through a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK).

Magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK). Single, paired or triple TMS pulses were delivered by a Magstim 200 machine, and rTMS was delivered using a Magstim Super Rapid stimulator connected to four booster modules. The coil was placed tangentially to the scalp with the handle pointing posteriorly to deliver the stimulation in all experiments. The optimal location of the coil was defined as the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral FDI when the subject was relaxed (the "motor

hot-spot"). The stimulation intensity was defined in relation to either rest motor threshold (RMT) or the active motor threshold (AMT) of the subject. The RMT was defined as the minimum stimulation intensity over the motor hot-spot that could elicit an MEP of no less than 50μV in five out of ten trials from the contralateral FDI. The AMT was defined as the minimum intensity of single pulse stimulation required to produce an MEP of greater than 200μV on more than five out of ten trials while the subject was maintaining a voluntary contraction of about 20% of maximum in the FDI. Visual feedback was provided to the subject to help maintain a constant muscle contraction of the correct force.

5.2.3 Experiments

5.2.3.1 The long interval inhibition of a pulse

In chapter 3, I have demonstrated that a short burst at 50Hz and 80% AMT transiently increases MEP amplitude and decreases short interval intracortical inhibition (SICI) for up to 100 ms followed by a longer lasting decrease in SICI (Fig 3.4) with a possible different mechanism. A similar phenomenon can be also found using a single pulse at higher intensity. A pulse at suprathreshold intensities can facilitate the MEP size evoked by a test pulse 10-40ms after it, whereas the test MEP was inhibited at interstimulus intervals in the range of 50 ms to approximately 200 ms (i.e. long interval intracortical inhibition, LICI). In the range where the MEP was suppressed, the SICI has been demonstrated to be decreased, and an interaction between SICI and LICI was suggested (Sanger et al., 2001). It is therefore reasonable to suspect that the decreased SICI at around 150 ms after a burst at 50 Hz could be

due to an inhibitory effect similar to LICI. However, due to safety concerns, I used a single pulse at subthreshold intensities to investigate the possibility of producing the inhibitory effect on SICI without changing the amplitude of MEPs, instead of using a burst at suprathreshold intensities to suppress MEPs at that period of time.

I assessed SICI at an interstimulus interval (ISI) of 2ms and ICF at ISI of 10ms using the double-pulse method described by Kujiari et al. (Kujirai et al., 1993) on nine subjects (7 men, 2 women; mean age, 30±6 years) in the motor hand area. The first pulse at an intensity of 110%, 90% or 80% RMT was given at 150ms before the test stimulus of paired pulses. I assessed SICI and ICF using a conditioning stimulus intensity of 90% AMT, and the test stimulus was set at an intensity that would evoke an EMG response of around 1 mV peak-to-peak amplitude, when given alone. These assessments were separated into three blocks of 60 trials with the different stimulation conditions given at variable intervals (4.5-5.5 sec) in a random sequence.

5.2.3.2 The effect of a single train of bursts

To better understand the mechanism of our different TBS paradigms, we explored the effect of a single train of 10 and 25 bursts given over the motor hand area. MEPs were accessed 4-5 seconds before the train of bursts and at 1 second, 5 seconds, 10 seconds, and 15 seconds after the train in one block of testing. The block was then repeated every 40-45 seconds for 10 repeats. Two separate sessions using either a 10 bursts or a 25 burst train were assessed in each subject. Five subjects (3 men, 2 women; mean age, 27±5 years) were recruited for this experiment.

5.2.4 Data analysis

Data were analysed using SPSS for Windows version 11.0. Repeated measures ANOVA was used to compare variables before and after a single train of bursts, and paired t-tests were used to compare the effect of a single pulse on the MEP size, SICI and ICF.

5.3 RESULTS

5.3.1 The long interval inhibition of a pulse

The results of MEP size are shown in figure 5.1A. A two-way ANOVA with CONDITION (with and without the first conditioning pulse) and INTENSITY (110%, 90% and 80% RMT)) as main factors showed a significant main effect of INTENSITY ($F(2,16)=4.97$, $p<0.05$). The MEP size was significantly decreased at 150ms by a pulse at 110% RMT ($t=4.46$, $p<0.005$), but not by a pulse at 90% or 80% RMT. Figure 5.1B shows the results of SICI. A two-way ANOVA with CONDITION (with and without the first conditioning pulse) and INTENSITY (110%, 90% and 80% RMT)) as main factors showed a significant main effect of CONDITION ($F(1,8)=13.12$, $p<0.01$), but no effect of INTENSITY ($F(2,16)=0.12$, ns). This finding was because the SICI was reduced by the first conditioning pulse at 110% or 90% RMT (110%: $t=-2.30$, $p<0.05$; 90%: $t=-5.01$, $p<0.001$) (Fig 5.1B). The ICF was not significantly modified (2-way ANOVA, ns), although it tended to be reduced by a pulse at 110% and 90% AMT (Fig 5.1C).

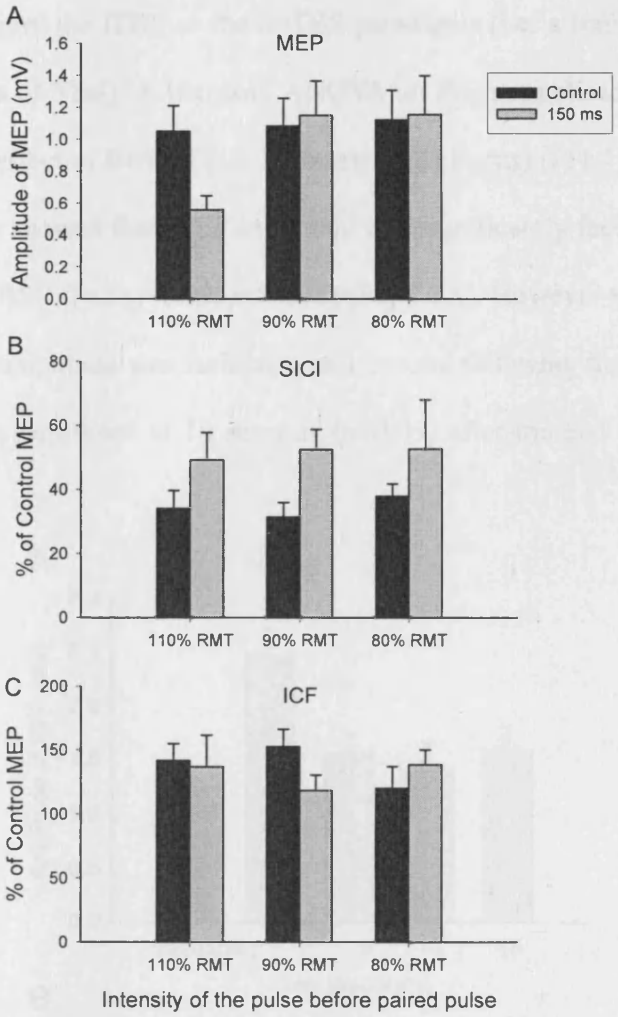

Fig 5.1 ***The effect of a pulse at different intensities on the amplitude of MEP, SICI and ICF at 150 ms after.*** *A, The amplitude of MEP was suppressed by a conditioning pulse at 110%, but not 90% or 80%, RMT. B, SICI was reduced by a pulse, even when it did not change the test MEP size. C, ICF was not significantly modified by a conditioning pulse.*

5.3.2 The effect of a single train of bursts

The results in chapter 4 show that a TBS protocol in which the bursts were applied in short trains (2s) (iTBS) induced facilitation, whereas a protocol in which the bursts were applied in a single continuous train induced inhibition. We explored this further by comparing the short-term effects on the amplitude of MEPs after applying just a

single train of either the iTBS or the imTBS paradigms (i.e. a train of 10 bursts of TBS or 25 bursts of TBS). A two way ANOVA on this normalised data revealed a significant main effect of BURST (i.e. 10 bursts or 25 bursts) ($F(1,4) = 9.07$, $p < 0.05$). Post hoc analysis showed that MEP amplitude was significantly facilitated by a train of 10 bursts of TBS ($F(4,16) = 6.99$, $p < 0.005$) (Fig 5.2A). However with a train of 25 bursts, the MEP amplitude was facilitated at 1 second following the end of the train ($p < 0.05$) but was depressed at 10 seconds ($p < 0.05$) after the end of the train (Fig 5.2B).

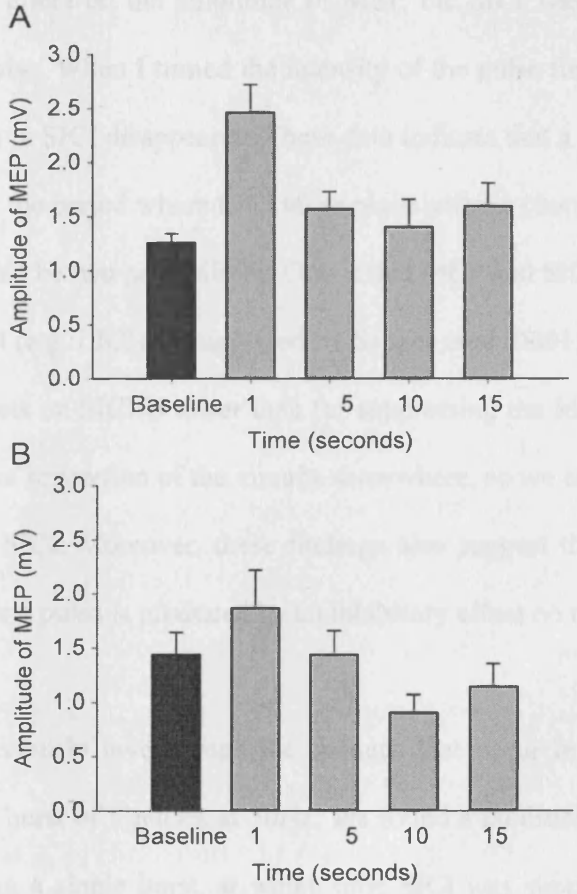

Fig 5.2 ***Illustrates the effect on MEP size of a short burst of TBS given for either 2s (Fig 2Aa) or 5s (Fig2Ab).*** *MEP size was measured at baseline and then at 1, 5, 10 and 15s following the end of stimulation. Following a 2s train of TBS (Fig 2Aa) there was a significant facilitation of MEP size (F(4,16)6.99, p<0.005)). In contrast a 5s train of TBS produced an initial significant facilitation of MEP size at 1 second after the end of stimulation (p<0.05) followed by a significant suppression of MEP size at 10s (p<0.05).*

5.4 DISCUSSION

5.4.1 A mixture of excitatory and inhibitory effects produced by the stimulation

A suprathreshold pulse reduced SICI at the interval where LICI was produced (Sanger et al., 2001). In this study, a pulse at 110% RMT inhibited the MEP through LICI at 150ms after the pulse. I then reduced the intensity of the pulse to 90% RMT, which showed no effect on the amplitude of MEP, the SICI was still decreased at 150ms after the pulse. When I turned the intensity of the pulse further down to 80% RMT, the decrease in SICI disappeared. These data indicate that a subthreshold pulse can reduce SICI at the period where LICI takes place without changing the amplitude of MEP. There could be two possibilities. One is that MEP and SICI were suppressed by the same circuit (e.g. LICI) as suggested by Sanger et al (2001), but the threshold for producing effects on SICI is lower than for suppressing the MEP size. The other is that there is some separation of the circuits somewhere, so we can see no effect on LICI yet reduced SICI. Moreover, these findings also suggest that the decrease in SICI at 150ms after a pulse is produced by an inhibitory effect on the SICI circuit..

We have previously investigated the changes that occur in the motor system following a single burst of 5 pulses at 50Hz. We found a facilitation of MEP size at 20-40 ms following a single burst, at which time SICI was suppressed. There was then a longer latency effect at 100-150ms following the burst where SICI was suppressed again, but MEP size was not altered. As described above, MEP size can be suppressed at 150ms after a single pulse, but only if the pulse is delivered at a sufficient intensity (e.g. 110% of RMT in this experiment). When the pulse is at a

lower intensity, only SICI will be suppressed. These results may suggest that the decreased SICI occurring at 100-150ms after a burst is caused by an inhibitory effect. However, this inhibitory effect is too weak to depress MEP size. Taken together, these data would suggest that a burst of rTMS produces an increase in motor system excitability which occurs early after the burst, and requires a low intensity of stimulation to be produced. There is subsequently a longer latency effect that occurs following a burst that is inhibitory in nature. This effect is weaker than the excitatory effect that occurs earlier, as a burst of sub-threshold intensity which can facilitate the MEP at 20-40ms, has no effect on the MEP at 150ms. There are therefore opposing effects on motor cortical excitability that occur after a single burst that have different strengths and time courses.

Data from animal studies using short trains of high frequency stimulation, would suggest that both excitatory and inhibitory effects can be summated to produce more powerful and longer lasting effects when pulses are given at a sufficiently high frequency. (Schmidt and Perkel, 1998; Beierlein et al., 2003) We hypothesise that the same phenomenon occurs after TBS magnetic stimulation. After conditioning with TBS in our experiments, we hypothesise that prolonged excitatory and inhibitory effects are induced. The overall effect on cortical excitability is determined by the dominant effect. As outlined above, there is evidence that excitatory effects after a TMS burst or single pulse are of a short latency and are more powerful than the longer-latency inhibitory effects (i.e. are more easily produced). When a stimulus is given in a fixed interval, the half-life of the effect it has determines how fast the accumulated effect will plateau and the ratio of half-life: interval determines the level of the plateau. The shorter the half-life, quicker the

effect will reach a plateau. The larger the half-life:interval ratio, the higher the level of the plateau. Therefore, the short half-life of this excitatory effect could make it plateau more easily after repeated stimulation. Although the inhibitory effect following a TMS burst may be weaker, if stimulation duration is sufficient then the longer half-life of the weaker inhibitory effect should allow it to continue to accumulate after the excitatory effect has reached a plateau, and to eventually overcome the initial dominant excitatory effect.

The experiments comparing the effect of applying a single train of TBS support this hypothesis. A 2s train (i.e. the individual component of the iTBS pattern) had a purely facilitatory effect on MEPs, whereas MEPs were initially facilitated after a 5s train (the component of the imTBS pattern), but then suppressed at 10s before returning to baseline at 15s. Given that a 20s train of TBS (i.e. the cTBS pattern) is purely suppressive, this suggests that a single train of TBS can lead to a mixture of suppressive and facilitatory effects on MEPs, with facilitation building up faster than suppression, but with suppression being more powerful in the long term.

There is also indirect evidence in support of this hypothesis from previous animal studies. When Larson et al. first developed the theta burst pattern of stimulation (Larson et al., 1986), they actually found that the LTP effect produced by stimulation was considerably smaller when 20 bursts were used compared with 10 bursts. Beierlein et al. (Beierlein et al., 2003) have reported an initial facilitation followed by depression during a train of stimulation at frequencies higher than 20 Hz, compatible with the theory that a shorter train of stimulation is excitatory while the longer train is inhibitory.

5.4.2 The mechanism

At first sight the opposing effects of different patterns of TBS are surprising. Since these experiments were performed on human subjects, it is only possible at present to speculate on the reasons underlying the difference in after-effects of cTBS and iTBS. However, a similar dissociation has been noted in previous work on animal preparations: patterns of intermittent TBS similar to our iTBS paradigm are routinely used to facilitate synaptic connections (Capocchi et al., 1992; Hess and Donoghue, 1996; Heynen and Bear, 2001), whereas a small number of studies have used longer trains of TBS-like paradigm to produce suppression (Takita et al., 1999; Heusler et al., 2000). If similar changes in synaptic efficacy are responsible for the effects we describe in the human motor cortex then it seems likely that cTBS reduces the transmission through the synaptic connections that are recruited when evoking an MEP whereas iTBS has the opposite effect. Similar arguments can account for the changes in SICI and ICF that we observed. Thus, cTBS reduced the excitability of both SICI and ICF; iTBS had the opposite effects on SICI although there was no significant effect on ICF. This may be because more than one circuit contributes to ICF, or that the time course of the changes is subtly different in the ICF circuit compared with that for MEPs and SICI.

The results of the experiments with single trains of TBS suggest that in humans TBS produces a mixture of facilitatory and inhibitory effects on synaptic transmission, with facilitation building up faster than inhibition. A simple model along these lines is developed below and illustrated in Fig 5.3. Such a simple model shows that by taking values of time constants from the present experimental data, it is possible to account for the opposite effects of cTBS and iTBS, and also to show

why imTMS has no overall after effect (the mix of inhibition and facilitation is equal). This model has clear parallels with data from physiological studies of the events occurring at synapses during plastic changes.

In a naïve synapse, as in our experiments, LTP induction appears to be associated with phosphorylation of calcium/calmodulin-dependent protein kinase II (CaMKII), while LTD induction is associated with dephosphorylation of cyclic-AMP-dependent protein kinase (PKA) site (Lee et al., 2000). A key factor in determining the occurrence and amount of phosphorylation/dephosphorylation is calcium. LTP is favoured when the postsynaptic calcium is at high levels, whereas moderate levels of postsynaptic calcium result in LTD (Malenka and Nicoll, 1999; Kemp and Bashir, 2001; Sheng and Kim, 2002). In fact, the temporal pattern of calcium increase may be even more important than the absolute level. Yang at al (1999) demonstrated that LTP is triggered by a sudden increase in $[Ca^{2+}]$, whereas a more prolonged modest rise of $[Ca^{2+}]$ reliably triggers LTD. Activation of inositol triphosphate receptors ($InsP_3Rs$) allows a slow release of Ca^{2+} from internal stores, favouring the production of LTD. A sudden influx of Ca^{2+} tends to desensitise these receptors.

Calcium can therefore influence the direction of change in synaptic efficiency depending on the concentration and time course over which it is released. This has clear parallels with our modelling of the mechanism behind the effects of different TBS paradigms. When TBS is given in short trains, as in the iTBS paradigm, it is possible that a large amount of Ca^{2+} influxes quickly, desensitizing InsP3Rs, promoting phosphorylation of CaMKII sites. After a short train, this may not reach the threshold for LTP and may instead cause a short-term potentiation (STP) effect

(Malenka and Nicoll, 1999). The level of [Ca^{2+}] is likely to decline quickly to baseline during the pause between trains. And when the next short train is given a new wave of Ca^{2+} influx produces more phosphorylation. After a few trains, the effects are summated to reach the threshold to cause LTP after the end of the stimulation. When the bursts are given continuously, as in the cTBS paradigm, the rate of Ca^{2+} influx decreases gradually over the course of stimulation. This could allow a recovery in function of the InsP3Rs, promoting a longer lasting moderate rise in [Ca^{2+}], therefore favouring dephosphorylation of PKA sites and the induction of LTD. When the length of a train is intermediate (as in the imTBS paradigm), the effects of the initial sharp rise in [Ca^{2+}] are matched by a degree of recovery in InsP3R function, producing a state of equilibrium between phosphorylation /dephosphorylation, and therefore LTP/LTD.

We therefore assume that each 3-pulse burst of stimulation causes an influx of Ca^{2+} at synapses (Fig 5.3 upper panel). This secondarily results in a build up of facilitatory or inhibitory "substances", with the former being proportional to the rate of increase in Ca^{2+} whereas the latter is related to the amount of Ca^{2+}. The time course of each is different, but the maximum amount of the inhibitory substance is greater than the facilitatory substance (Fig 5.3 second row). At the end of a series of trains, the final amount of each substance then interacts with a second process that causes the long term after effects that persist over many minutes after the end of the period of stimulation (Fig 5.3 third row). This second process builds up over several minutes, with a time course that depends on the pattern of TBS and ratio of facilitatory to inhibitory effects, and then decays slowly.

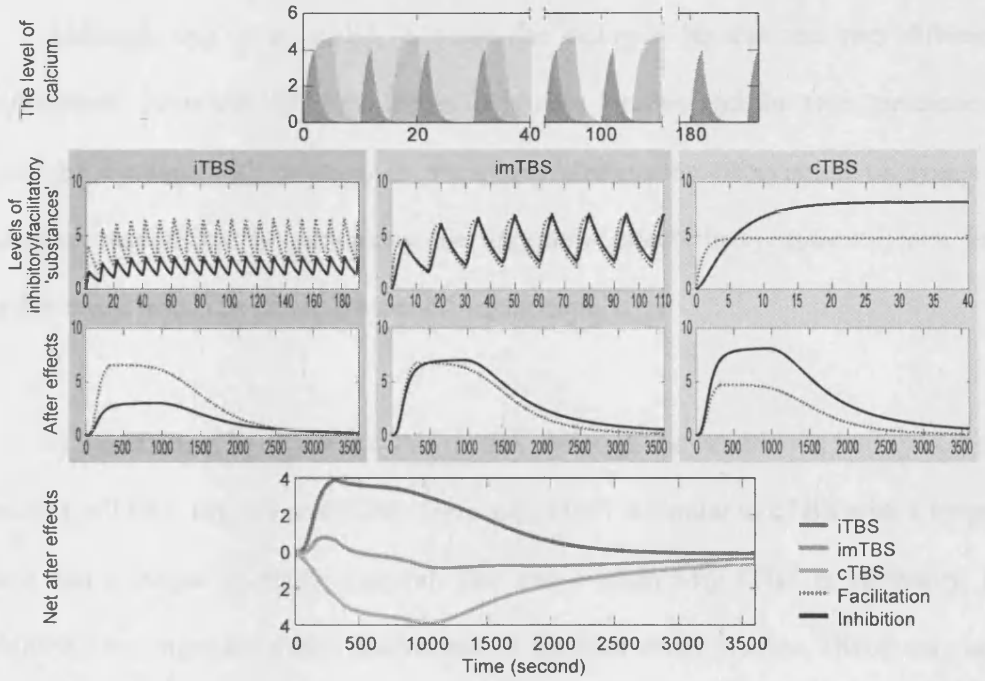

Fig 5.3 ***Results of a simple model that accounts for the different long lasting effects of cTBS, imTBS and iTBS.*** *The model has three stages, represented by the three rows of graphs. In the first stage TBS causes an increase in the postsynaptic concentration of Ca^{2+}. With cTBS (light grey shading), Ca^{2+} rises rapidly to a peak and then remains at that level until the end of the conditioning (40s). With iTBS (dark grey) and imTBS (grey), the Ca^{2+} initially rises in the same way but then declines exponentially at the end of each train (i.e. after every 2 s train for iTBS, and after every 5s train of imTBS). In the second stage, the Ca^{2+} interacts with a second process that produces facilitatory and inhibitory "substances". These could be, for example, different levels of protein kinases in the postsynaptic neurone. The facilitatory "substance" rises at a rate proportional to the rate of increase in Ca^{2+}, whereas the inhibitory "substance" rises more slowly proportional to the level of Ca^{2+}. Thus during cTBS (rightmost graph), the initial rapid rise in Ca^{2+} causes a rapid increase in the facilitatory "substance" (dot line), but this remains constant thereafter. The sustained increase in Ca^{2+} during cTBS causes a slower rising but larger increase in the amount of the inhibitory "substance" (black line). With iTBS (leftmost graph), the rapid increases in Ca^{2+} at the start of each 2s train cause a greater production of facilitatory than inhibitory "substances". With imTBS, the amount of each type of "substance" is equal. In the third stage (third row), the final level of the two "substances" interacts with two corresponding slower processes that may be analogous to phosphorylation/dephosphorylation of membrane bound ion channels responsible for production of LTP/LTD. They rise and fall with sigmoid time courses. Finally (bottom row), the net effect on MEP amplitudes is modelled as the sum of these positive and negative after effects. Following cTBS, suppression is larger than facilitation and the MEPs are suppressed for many minutes. The opposite occurs after iTBS, whilst after imTBS, suppression and facilitation are matched and there is virtually no net effect on MEP amplitudes.*

Although this is a model, it could for example be that the two different "substances" represent different levels of protein kinases and the two "processes" could be equated with changes in phosphorylation/dephosphorylation of synaptic receptors responsible for producing the long term effects in synaptic efficacy, but further work would be needed to confirm such details.

sTBS can also be fitted into this model, although the model was built based on the data of iTBS, imTBS and cTBS. Basically, sTBS is similar to cTBS with a longer burst and a longer interburst interval. This can explain why sTBS is inhibitory. In addition, the longer interburst interval makes the after-effect weaker. This theory can be also applied to explain the effects of traditional rTMS paradigms. It is usually suggested that a significant inhibitory effect can be produced after 10 minutes or more 1Hz rTMS conditioning. (Chen et al., 1997; Touge et al., 2001) An increase in excitability is often reported with rTMS using frequencies of 5Hz. This is in contrast to animal data where long trains (20 minutes) of stimulation at 1 or 5Hz can produce reliable LTD effects. The difference between these animal and human studies is that due to safety concerns and technical problems with coil overheating, 5Hz stimulation is typically given in single short trains (Berardelli et al., 1998; Maeda et al., 2000; Wu et al., 2000) or more commonly in repeated short trains (Siebner et al., 1999; Gilio et al., 2002). It is therefore possible that the opposite effect of the 1Hz and 5Hz rTMS paradigms on cortical excitability in human studies is due to the pattern of stimulation (continuous vs. intermittent) rather than the frequency.

5.4.3 Mathematical modelling

We have devised a simple theoretical model to describe the results of the different

types of TBS. This has three stages, each related to a known process that has been shown to occur in one or more types of LTP or LTD. In the first stage, we assume that the bursts of 3 stimuli at 50 Hz each result in postsynaptic Ca^{2+} influx that decays exponentially after each burst. When the peak level of Ca^{2+} after a burst is C, the effect at a time point (t) after the peak level will be

$$C(t) = C \cdot e^{-kt} \tag{1}$$

When bursts are given regularly at t minutes after the peak level, the maximum level of Ca^{2+} after n bursts will be

$$C^n_{max} = C \cdot (1 + e^{-kt} + e^{-2kt} + ... + C^{-(n-1)kt}) = C \cdot \frac{1 - e^{-nkt}}{1 - e^{-kt}} \tag{2}$$

Whereas the minimum level after n bursts becomes

$$C^n_{min} = C^n_{min} \cdot e^{-kt} = C \cdot \frac{1 - e^{-nkt}}{1 - e^{-kt}} \cdot e^{-kt} \tag{3}$$

In the second stage we propose that the Ca^{2+} influx leads to production of a "facilitatory" or an "inhibitory" substance designed to be equivalent to activation of different types of protein kinases. In the model, the "facilitation" accumulates according to the rate of increase in Ca^{2+}, whereas "inhibition" accumulates more slowly according to the overall level of Ca^{2+}. Both decay exponentially with time.

In the last stage of the model, these substances interact with a process that leads to long term changes in synaptic effectiveness. These may be equivalent to phosphorylation or dephosphorylation of AMPA receptor proteins giving rise to LTP and LTD respectively. The effects build up over minutes after the end of TBS with a sigmoidal profile and decay much more slowly, again with a sigmoidal profile. The time course was modelled as follows. If the maximum effect of the conditioning is M

and this occurs at time (t_{peak}), then the time from onset to reach half of the maximum effect is $t_{50(o)}$, while the time taken to decline to the half of the maximum is $t_{50(d)}$. Thus the effect at a time point (t) after the TBS will be

$$M(t) = \frac{M \cdot t^{h1}}{t_{50(o)}^{h1} + t^{h1}} \quad (t \leq t_{peak}) \tag{4}$$

$$M(t) = \frac{M \cdot t_{50(d)}^{h2}}{t_{50(d)}^{h2} + t^{h2}} \quad (t > t_{peak}) \tag{5}$$

Where *h1* and *h2* are time constants that describe the steepness of the sigmoid curves. The final result is modelled as the sum of the inhibitory and facilitatory effects, and represents the time course of the long term changes in MEP that we observe experimentally.

Parameters for modelling Ca^{2+} changes

First of all, we set C as 1. Because a 3-pulse burst at 50 Hz takes around 40 ms and a burst was given every 200 ms, the t for calculating the decayed effect at the time when the next burst comes is 0.16 seconds. We set the decay constant k to be 1.2. This value is somewhat arbitrary since the model is relatively insensitive to values of k within a very large range.

Parameters for cascades of substances for LTP or LTD

Most parameters in this mathematical model are based on results of this experiment or the experiments examining the effect of a single burst. Those which could not be obtained from original data were estimated.

The facilitatory "substance" was modelled as accumulating proportional to the rate of the increase of Ca^{2+}. The proportionality constant was set to 1. The exact

value of this is again arbitrary since the overall effect of the model depends on the ratio between this value and that for the inhibitory "substance". The latter was modelled as being related to the steady level of $[Ca^{2+}]$ with a proportionality constant of 0.07. These two constants for facilitation and inhibition ensured that after 5 single train of 5s TBS, the amount of each "substance" would be approximately equal. Both substances decayed exponentially between bursts with the inhibitory substance decaying more slowly than the facilitatory substance (time constants of 0.8 and 1.0 respectively).

Parameters for modelling the effect after TBS

The maximum effect, *M*, is the maximum level calculated above for the facilitatory or inhibitory "substances". To estimate the time of t_{peak} after a TBS, we used a sigmoid curve to fit the known data, in which the peak inhibitory effect was 0.15 seconds after a burst, 5 seconds after 25 bursts, 7 minutes after 100 bursts, and 16 minutes after 200 bursts. These times were chosen according to the time of peak after effects on MEPs after different durations of cTBS. We used the following function to estimate the t_{peak} for an arbitrary number of bursts (*burstnumber*)

$$t_{peak} = \frac{1200 \cdot burstnumber^{3.2}}{130^{3.2} + burstnumber^{3.2}} \qquad (6)$$

We set t_{peak} of excitation to be around one third of t_{peak} of inhibition, because after 200 bursts, the best MEP facilitation occurred 5 minutes after iTBS while the best suppression occurred at around 15 minutes. The parameters for the onset and decline sigmoid curve were chosen by fitting the simulated results with the results of experiments. For the excitatory effect, *h1* was set to 3.5 and *h2* was 4. The $t_{50(o)}$ was modelled as a half of the t_{peak} and the $t_{50(d)}$ was four times t_{peak}. For the inhibitory

curves, the *h1* was set to 3 and the *h2* was 2. The $t_{50(o)}$ was 0.18 times t_{peak} and the $t_{50(d)}$ was 0.8 times t_{peak}.

5.5 CONCLUSIONS

A pulse or a burst of stimulation can induce a shorter and stronger excitatory effect followed by a slower and weaker inhibitory effect. The mixture of facilitatory and inhibitory effects means that the direction of the effect can be changed by modifying the pattern of the conditioning stimulus without changing the intensity or frequency of the stimulation.

Chapter 6 Modulating the effect of TBS by physiological activity

The phenomena of long-term potentiation (LTP) and long-term depression (LTD) can be induced by electrical stimulation and have for many years been used as a model of neuroplasticity in animal preparations. However, they are difficult to obtain in the cerebral cortex of freely moving animals so that their interaction with normal behaviours has been rarely studied. In chapter 4 I introduced a new method of producing a rapid and reliable long term change in human motor cortex excitability with theta burst stimulation (TBS). Although there is no direct evidence, it is possible that part of the long term effects that it produces on cortical excitability depend on processes of LTP and LTD. If so then it may be possible to use this method to investigate interactions between mechanisms of plasticity and behaviour in the human brain.

In chapter, I shall show that the physiological activity produced by voluntary muscle contraction interacts with LTP/LTD-like effects induced by TBS. The tentative conclusion is that physiological activity, which alters the distribution of cortical excitability, interacts with processes that produce LTP and LTD at cerebral synapses in the conscious human brain.

6.1 INTRODUCTION

It has long been known that LTP and LTD induced by electrical stimulation of nervous pathways in animal preparations can be reversed by patterns of stimulation that on their own have no long term effects on synaptic transmission. These effects

are known as de-potentiation and de-depression respectively. For example, LTP can be reversed in hippocampus by a low frequency train of stimulation at 1-2 Hz (Chen et al., 2001; Huang et al., 2001; Huang et al., 2002), particularly if the latter is applied within minutes of the end of the induction of LTP. More recently, there have a number of reports that LTP can also be reversed by natural patterns of activation in conscious animals. Thus electrically elicited LTP in hippocampus was reversed in rats when they entered a new environment, particularly if this was done within 1 hour of onset of LTP (Xu et al., 1998; Manahan-Vaughan and Braunewell, 1999). Exploration of the new environment induced 6-8Hz activity in the whole hippocampal network, and this is thought to have this led to de-potentiation of LTP. A similar effect was observed on LTP at retinotectal synapses after exposure to a visual stimulus (Zhou et al., 2003). Zhou and Poo (2004) suggested that this type of effect could enhance the emergence of connectivity based on patterned inputs and reduce the error in activity-dependent circuit refinement resulting from random inputs and activities (Zhou and Poo, 2004).

The behaviour of conscious humans is very easy to modify by prior instruction and hence in many ways, humans are much more suitable than other animals to be subjects in experiments designed to investigate the influence of physiological activities on synaptic plasticity. However, this depends on the existence of an optimal tool to produce reliable effects on synaptic plasticity in human subjects. To date, various forms of rTMS have been proposed to produce LTP and LTD-like effects in human motor cortex, hence these may be a useful practical way of investigating interactions with physiological activity. In this chapter I will describe how the new method of theta burst stimulation is affected by voluntary muscle contraction. I will show that the results are consistent with the idea that physiological activity in the

brain interacts with LTP- and LTD-like effects in a way very similar to that in animal experiments. I propose that the effects are caused by voluntary activation of neuronal networks in the motor cortex in ways that de-potentiate or de-depress the changes in synaptic effectiveness evoked by TBS.

6.2 METHODS AND SUBJECTS

6.2.1 Subjects

All subjects for these experiments were healthy volunteers between the ages of 23 and 43 (mean age: 30.9±6.8 years) and gave their informed consent for the experiments. The project protocol was approved by the Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery.

6.2.2 Stimulation and recording

For all experiments, subjects were seated in a comfortable chair. EMGs were recorded using Ag-AgCl electrodes from the right first dorsal interosseous (the dominant hand in all subjects). EMG activity was recorded with a gain of 1000 and 5000 and filtered with a band-pass filter (3 Hz to 2k Hz) through Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK).

Magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK). Single and paired pulses were delivered by Magstim 200 machines, and rTMS was delivered

using a Magstim rapid stimulator connected to four booster modules. Stimulation was delivered over the motor hand area with the coil placed tangentially to the scalp with the handle pointing posteriorly. The motor hand area was defined as the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral first dorsal interosseous (FDI) when the subject was relaxed (the "motor hot-spot"). The stimulation intensity was defined in relation to the active motor threshold (AMT) of the subject. The AMT was defined for each Magstim machine separately as the minimum intensity of single pulse stimulation required to produce an MEP of greater than 200μV on more than five out of ten trials from the contralateral FDI while the subject was maintaining a voluntary contraction of about 20% of maximum in the FDI. Visual feedback was provided to the subject to help maintain a constant muscle contraction of the correct force.

Electrical nerve stimuli were supplied by a constant current generator (Digitimer, UK).

6.2.3 Experiments

Single pulse TMS was used to evaluate the effect of TBS on MEPs elicited in the contralateral FDI. The intensity of these "probe" TMS pulses was set at an intensity that produced MEPs of 1mV in control conditions, unless described separately. MEP size, SICI and ICF were always assessed with the subject relaxed.

6.2.3.1 The TBS paradigms

As in chapter 4, the basic TBS pattern was a burst containing 3 pulses of 50Hz

magnetic stimulation given every 200ms (i.e. at 5Hz). Two different stimulation paradigms were used in this experiment: 1) Intermittent theta burst stimulation (iTBS): the basic pattern given in a short train lasting 2 seconds (i.e. 10 bursts), repeated every 10 seconds for 20 cycles. 2) Continuous theta burst stimulation (cTBS): the basic pattern given in a continuous train lasting 20 seconds (i.e. 100 bursts). TBS was delivered to the motor hand area at an intensity of 80% AMT.

Baseline MEP recording was performed using 30 pulses delivered every 4.5-5.5 seconds. TBS was then given with the subject relaxed (TBS-relaxed), and MEP size was assessed using single pulses of TMS delivered in trains of 12 pulses given every 4.5-5.5 seconds every 1 minute for 6 minutes, then every 2 minutes until 23 minutes after the end of TBS. Nine subjects (6 men, 3 women; mean age: 31±8 years) participated in the TBS experiment.

6.2.3.2 Contraction during TBS conditioning (cTBS-contract or iTBS-contract)

TBS was given with the subject performing a voluntary contraction of the FDI contralateral to the site of stimulation. This voluntary contraction was maintained throughout the period of TBS at about 10% of maximal force with visual feedback provided to the subject to encourage a constant force of contraction throughout stimulation. MEP size was tested on eight (5 men, 3 women, mean age: 32±7 years) of the nine subjects in cTBS-contract and seven of them (5 men, 2 women; mean age, 32±5 years) in iTBS-contract, and SICI and ICF were tested on seven subjects (4 men, 3 women; mean age, 27±3 years) in the motor hand area before and after TBS-contract. We assessed SICI at an interstimulus interval (ISI) of 2ms using a

conditioning intensity of 80% AMT, and ICF at an ISI of 10ms with a conditioning intensity of 90% AMT. We adjusted the intensity of the test stimuli while assessing SICI and ICF after TBS to maintain the amplitude of test MEPs at approximately 1 mV. MEP size, SICI and ICF were assessed in separate blocks. Two blocks of baseline MEP, SICI and ICF were recorded. After iTBS, MEP size, SICI and ICF were recorded every four minutes until 20 minutes following conditioning. After cTBS, MEP size and SICI was recorded every five minutes until 25 minutes after conditioning, while ICF was only recorded at 10 minutes, where we found the peak effect of cTBS to occur, and then at 30 minutes after cTBS.

6.2.3.3 Contraction immediately after TBS (cTBSc0 or iTBSc0)

In this part, subjects were asked to perform a voluntary contraction of the FDI muscle contralateral to the site of stimulation for 1 min immediately after TBS-relaxed. This voluntary contraction was maintained throughout the period of 1 min at about 10% of maximal force with visual feedback provided to the subject. Baseline MEP recording was performed using 30 pulses delivered every 4.5-5.5 seconds. TBS was then given and followed by the 1-min contraction. MEP size was assessed using single pulses of TMS delivered in trains of 12 pulses given every 4.5-5.5 seconds every 1 minute for 5 minutes, then every 2 minutes until 23 minutes after the end of TBS. Same nine subjects participated in the cTBS experiment, and only 7 of them (5 men, 2 women; mean age, 32±6 years) participated in the iTBS experiment. MEP size was always assessed with the subject relaxed.

We also did a control study using sham cTBS followed by the 1-min contraction in 7 of the 9 subjects (5 men, 2 women; mean age, 31±7 years). The

protocol is exactly the same as cTBSc0, except cTBS was replaced by a sham stimulation using a coil tilted 90 degrees away from the scalp.

In addition, we assessed SICI using a similar method to that described above before and after cTBSc0 on seven subjects (5 men, 2 women; mean age, 26±5 years). We adjusted the intensity of the test stimuli after conditioning to maintain the amplitude of test MEPs at approximately 1 mV. Two blocks of baseline SICI and ICF were recorded. SICI and ICF were recorded every 5 minutes until 25 minutes following conditioning.

6.2.3.4 Contraction at 10 min after the end of cTBS (cTBSc10 only)

The time course of changes in MEP size elicited from the contralateral FDI was measured using a similar method to that described in TBS paradigms on the same nine subjects. The only difference is that the subjects were requested to perform a voluntary contraction of the FDI contralateral to the site of stimulation at 10 min after the end of cTBS-relaxed for 1 min (cTBSc10). This voluntary contraction was maintained throughout the period of 1 min at about 10% of maximal force with visual feedback provided to the subject. Other than the minute when subjects were asked to activate the FDI muscle, subjects were relaxed for the MEP assessment.

6.2.3.5 cTBS followed by 1 min peripheral stimulation mimicking the contraction

The protocol of this experiment is exactly the same as the one described in the session of 'contraction immediately after TBS', except that the voluntary contraction

was replaced by a 1-min electrical stimulation on the ulnar nerve at wrist of the conditioned hand. Stimulation duration of an electrical pulse was 500μs, and the intensity used was that which produced the same amplitude of the voluntary contraction at about 10% of maximal force in FDI muscle. One thousand ulnar nerve stimuli were given in the minute (i.e. 17 Hz). Seven of the nine subjects (5 men, 2 women; mean age, 31±7 years) participated in this part.

6.2.4 Data analysis

Data were analysed using SPSS for Windows version 11.0. Repeated measures ANOVA was used to compare variables before and after TBS. Statistics for the data were performed on absolute amplitude values rather than the normalised amplitudes that are plotted in the graphs.

6.3 RESULTS

6.3.1 Application of TBS at rest compared with during voluntary contraction

Fig 6.1 compares the after effects of applying TBS at rest with TBS given during a static voluntary contraction of FDI. The upper panel shows the effects of iTBS, and the lower panel the data from cTBS. At rest, iTBS facilitated MEPs, as described in chapter 4; cTBS suppressed MEPs. However, if TBS was applied during contraction, then neither iTBS nor cTBS had any effect on MEP amplitude. This was confirmed in separate two factor ANOVAs for iTBS and cTBS. There was a significant TIME x CONTRACTION interaction for both the iTBS ($F(5,30)=2.92$, $p<0.005$) and cTBS

data ($F(5,40)=5.91$, $p=0.000$), indicating that TIME had a different effect on MEPs according to the state of muscle contraction when TBS was applied.

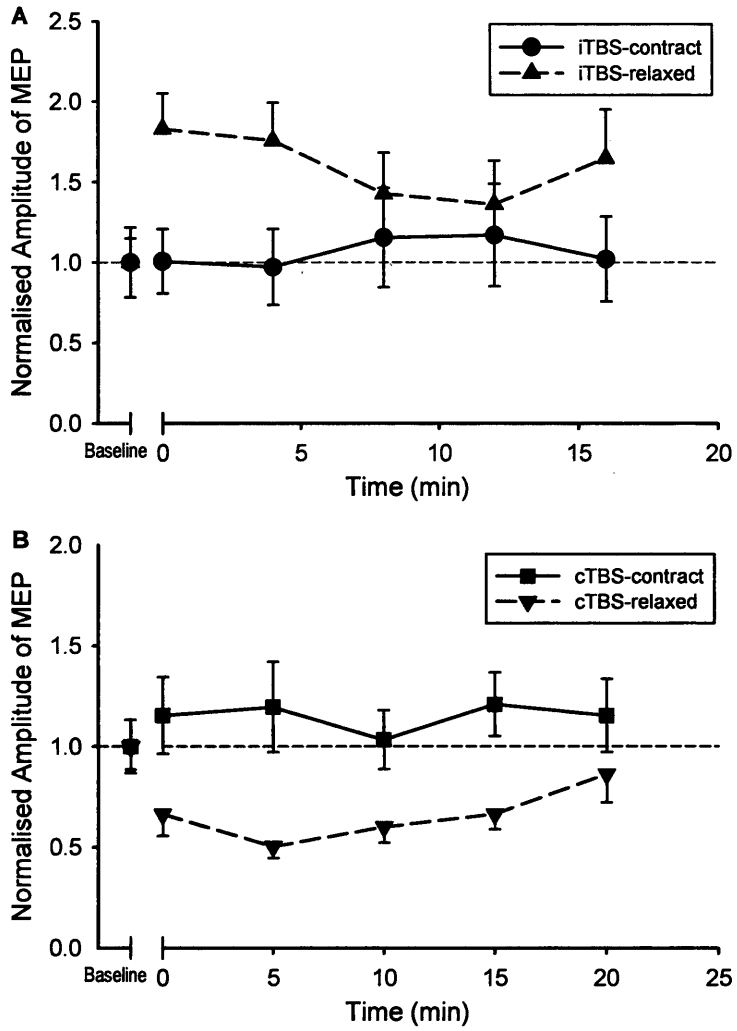

Fig 6.1 ***The time course of changes in MEP size following conditioning with iTBS and cTBS performed with target muscle relaxation or contraction.*** *(A) iTBS conditioning: (●) conditioning given during voluntary contraction of the FDI muscle; (▲) conditioning applied during muscle relaxation. MEPs were facilitated only if iTBS was given during muscle relaxation. (B) cTBS conditioning (■)conditioning given during voluntary contraction, (▼)conditioning during muscle relaxation. MEPs were suppressed only if cTBS was given during muscle relaxation.*

Separate one factor ANOVAs showed that MEPs were enhanced by iTBS-relaxed for up to 20 min ($F(15,120)=2.79$, $p=0.001$), whereas they were

suppressed by cTBS-relaxed for 20 min ($F(15,120)=4.35$, $p<0.001$). No significant effects were noted on MEP size at any time point if TBS was applied during contraction (cTBS; $F(5,30)=1.37$, *ns*: iTBS; $F(5,45)=1.00$, *ns*).

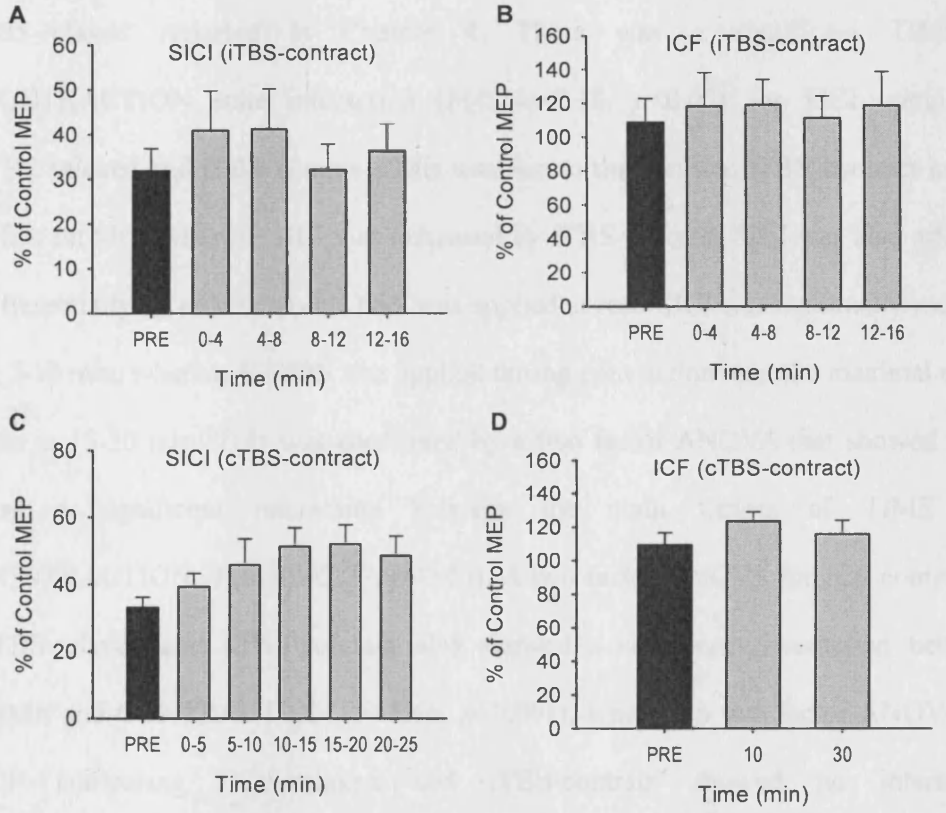

Fig 6.2 ***Effect of iTBS-contract and cTBS-contract on intracortical inhibition (SICI) and facilitation (ICF).*** *(A) SICI was not significantly changed following iTBS-contract (f(4,24)=0.78, ns). (B) ICF was not significantly altered following iTBS-contract (F(4,24)=0.203, ns). (C) SICI was significantly reduced following cTBS-contract (F(5,30)=5.83, p<0.001). (D) ICF was not changed after cTBS-contract.*

Fig 6.2a, b shows the data for SICI or ICF following iTBS performed during voluntary contraction. There was no significant change in SICI ($F(4,24)=0.78$, *ns*) or ICF ($F(4,24)=0.203$, *ns*) following iTBS performed during voluntary contraction. Fig 6.2c,d shows that cTBS, given with the subjects actively contracting, reduced the amount of SICI for the following 20min or more (one factor ANOVA on the time

course: $F(5,30)=5.83$, $p=0.001$), but that it had no significant effect on ICF at 10 and 30 min after cTBS.

We also compared the present data with the SICI and ICF results obtained with TBS-relaxed reported in Chapter 4. There was a significant TIME x CONTRACTION state interaction ($F(4,24)=3.38$, $p<0.05$) for SICI comparing iTBS-relaxed and iTBS-contract. This was due to the fact that iTBS-contract has no effect on SICI whereas SICI was enhanced by iTBS-relaxed. SICI was also affected differentially by cTBS: when cTBS was applied at rest, SICI was maximally reduced at 5-10 min, whereas if cTBS was applied during contraction then the maximal effect was at 15-20 min. This was confirmed by a two factor ANOVA that showed there was a significant interaction between the main factors of TIME and CONTRACTION ($F(5,30)=2.77$, $p<0.05$). A two factor ANOVA for ICF comparing cTBS-relaxed and cTBS-contract also showed a significant interaction between TIME and CONTRACTION ($F=8.66$, $p=0.001$), whereas a two factor ANOVA for ICF comparing iTBS-relaxed and iTBS-contract showed no interaction ($F(5,30)=0.80$, *ns*).

6.3.2 Contraction immediately after TBS (iTBSc0 and cTBSc0)

Fig 6.3a shows the results of iTBS-relaxed and iTBS followed immediately by a 1-min contraction. It appears as if the after effect of iTBS lasted longer when it was followed by contraction. This was confirmed in the ANOVA: there was a significant interaction between iTBS-relaxed and iTBSc0 ($F(14, 84)=2.45$, $p<0.01$). The MEP was persistently enhanced for more than 23 min ($F(14, 84)=3.55$, $p<0.001$) by

iTBSc0, whereas the effect of iTBS-relaxed only lasted for around 20 min ($F(15,120)=2.79$, $p=0.001$) and had a non-significant period between 9 to 15 min after the end of iTBS.

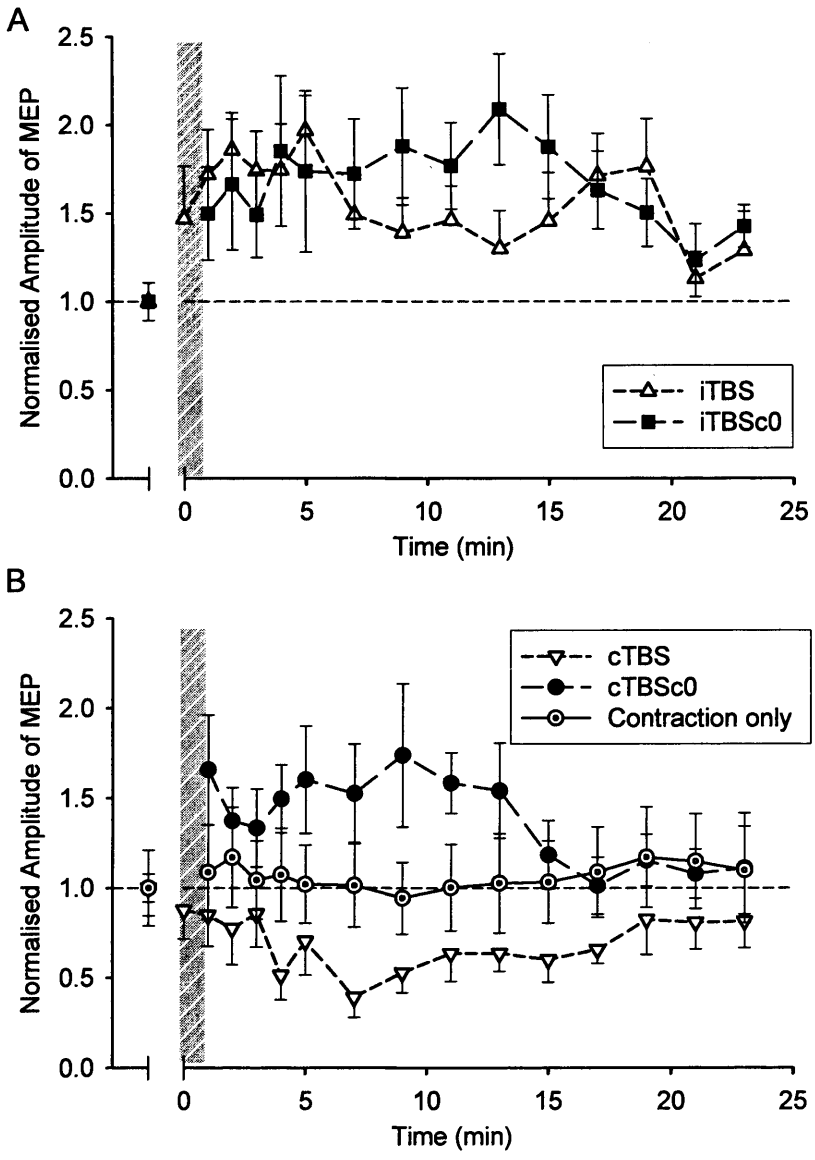

Fig 6.3 ***The effect of voluntary contraction immediately after TBS conditioning.*** *A) the facilitatory effect of iTBS was enhanced by the contraction. B) The contraction immediately after cTBS made the after effect opposite, while the contraction only had no after-effect*

Fig 6.3b compares the amplitude of MEPs elicited before and after cTBS-relaxed, cTBSc0 or contraction after sham cTBS. Surprisingly cTBSc0

reversed the effect of cTBS from inhibition to facilitation whereas sham TBS followed by contraction had no significant effect on MEPs (one factor ANOVA: $F(14,84)=0.971$, *ns*). Separate two factor ANOVAs showed that there was a significant interaction between cTBS-relaxed and cTBSc0 and also between cTBSc0 and contraction only ($F(14,98)=3.60$, $p<0.001$; $F(14,84)=1.96$, $p<0.05$, respectively). A one way ANOVA on the data from cTBSc0 showed that the facilitatory effect on MEPs was highly significant ($F(14, 98)=2.47$, $p=0.005$), and lasted for around 15 min.

Different from cTBS-relaxed (two factor ANOVA, TIME x CONTRACTION interaction ($F=2.40$, $p<0.05$)), cTBSc0 did not change the amount of SICI (One factor ANOVA on the time course: $F(5,30)=0.47$, *ns*) (Fig 6.4).

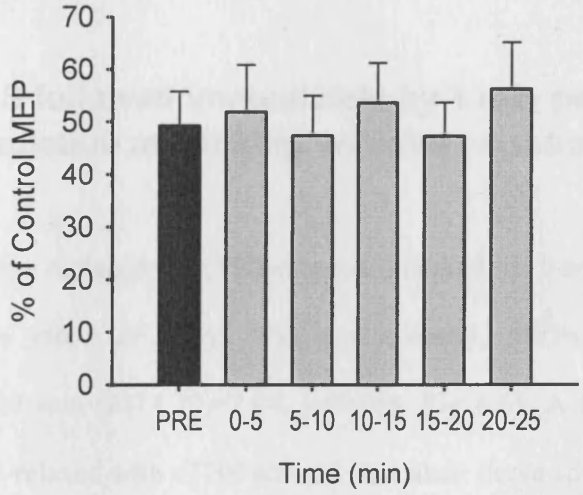

Fig 6.4 ***The effect of contraction immediately after cTBS on SICI.*** *cTBS followed immediately by contraction did not change the amount of SICI*

6.3.3 Contraction 10 min after the end of cTBS (cTBSc10)

Fig 6.5 compares the effect of cTBSc10 with cTBS-relaxed. A two way ANOVA showed there was a significant interaction between the main factors of

TIME and CONTRACTION ($F(15,120)=2.64$, $p<0.005$). Post hoc paired t-tests on normalised data showed that it was due to the first minute after contraction.

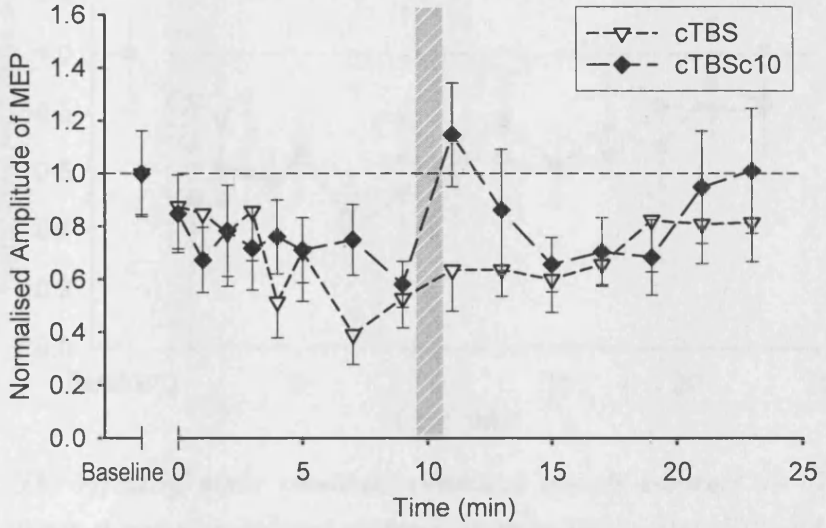

Fig 6.5 ***The effect of contraction at 10 min after cTBS.*** *The suppression effect of cTBS was only transiently reversed by the contraction at 10 min after conditioning.*

6.3.4 cTBS followed immediately by 1 min peripheral stimulation mimicking voluntary contraction

When cTBSc0 was replaced by cTBS-relaxed followed by 1-min simulation of the ulnar nerve, the effect of cTBS was not reversed. MEPs were significantly suppressed for 20 min ($F(14,70)=2.04$, $p<0.05$) (Fig 6.6). A two factor ANVOA comparing cTBS-relaxed with cTBS-relaxed plus ulnar nerve stimulation showed no significant TIME x GROUP interaction or a main effect of GROUP.

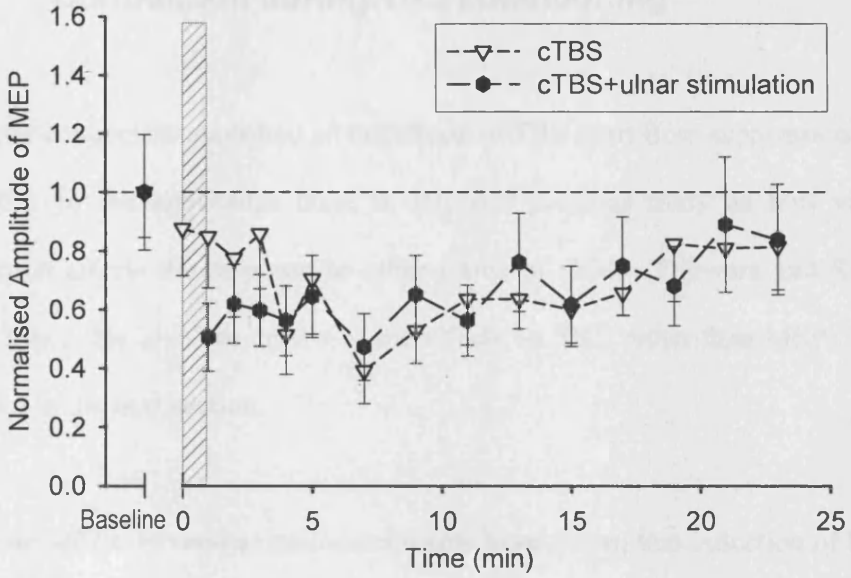

Fig 6.6 ***The effect of ulnar stimulation-induced muscle contract on cTBS.*** *The ulnar stimulation-induced contraction immediately after cTBS did not alter the after-effect of cTBS.*

6.4 DISCUSSION

The present results show that 1min voluntary contraction has a profound influence on the size and direction of the after-effects produced by TBS. Contraction *during* TBS abolishes all effects on the MEP and ICF, although effects on SICI remain. Contraction *immediately after* TBS improves the facilitatory effect of iTBS and reverses the inhibitory effect of cTBS from suppression to facilitation. Contraction *10min after* cTBS (iTBS was not tested) reduced MEP suppression, but only for 3-4min. All of these effects are compatible with the hypothesis that the after effects of TBS are due to LTP- and LTD-like effects on transmission at cortical synapses. As we argued in Chapter 4, the very low intensity used for TBS makes it unlikely that any of the after effects were due to changes in excitability at the spinal cord level.

6.4.1 Contraction during TBS conditioning

Voluntary contraction abolished all the effects of TBS apart from suppression of SICI by cTBS. To our knowledge there is only one previous study on how voluntary contraction affects the response to other forms of rTMS (Fujiwara and Rothwell, 2004). Since this involves details of the effects on SICI rather than MEPs, we will discuss it in the next section.

Effects on MEPs. Several animal experiments have shown that induction of LTP and LTD in the hippocampus can be affected by exposing animals to stress (Kim et al., 1996; Xu et al., 1997; Manahan-Vaughan, 2000); production of LTP is impaired whereas LTD is facilitated. However, they differ from the present experiments in that the stress was started before induction of LTP/LTD, and lasted up to 30 min after conditioning. Our voluntary contraction began at the start of TBS and lasted only while TBS was being applied. The result was that neither form of TBS (iTBS or cTBS) had any effect on MEP amplitude.

We would like to put forwards two hypotheses to account for our results. First, it may be that, as in many animal experiments, our LTP- and LTD-like effects are sensitive to the membrane potential of post-synaptic neurones. For example, an LTP-induction protocol, which produced LTP when the postsynaptic membrane was at the usual resting potential (-70 mV), led to LTD when the membrane was hyperpolarized to -85 mV (Randic et al., 1993). In addition, a 2 Hz stimulation for 50 seconds induced LTP, LTD, or nothing when the postsynaptic membrane potential was > -20 mV, between -20 and -40 mV, or ≤ -40 mV, respectively (Ngezahayo et al., 2000). It seems possible that a similar effect could have occurred in the present

experiments: that during contraction, the membrane potential of post-synaptic neurones changed, and this affected the response to the conditioning protocol. Precisely which post-synaptic neurones these might be is unknown. We can only suggest that they must lie in the pathway that leads to I-wave input to corticospinal neurones, since it is the excitability of this pathway that is tested when we measure MEP amplitude.

A second possible explanation is that contraction activates the same set of synaptic connections as those stimulated by TBS. In effect, this could cause a "busy line" effect, in which the extra activation evoked by TBS was negligible. Alternatively, if these pathways could still respond to TBS, it might be that synaptic activity caused by voluntary contraction changed the level of Ca2+ in post-synaptic neurones, and that this interfered with the cascades responsible for LTP and LTD.

Whatever the mechanism, the data are relevant to induction of synaptic plasticity in the neocortex of freely moving animals. This is much more difficult than induction in brain slices or anesthetised animals, and usually requires multi-sessions or extended protocols to produce a stable effect (Trepel and Racine, 1998; Froc et al., 2000). It may well be that part of this difficulty is due to unregulated amounts of synaptic activation in conscious animals. If so, it may turn out that the human cortex is a much better model for exploring this type of effect, given the ease with which we can control behaviour by simple instruction.

Effects on SICI/ICF. The effect on ICF was the same as on the MEP: both were abolished by contraction during TBS. Since both are net excitatory events, both

could be recruited during contraction and affect LTP- and LTD-like processes in the same way.

The effects on SICI were different. The most likely explanation for this is that contraction of FDI is accompanied by reduced excitability (and perhaps activity) in the SICI pathway to FDI (Ridding et al., 1995), rather than the increase in excitability of the MEP and ICF pathways. In analogy with the explanation above for MEPs, we might imagine that the membrane potential of SICI neurones was either unaffected or hyperpolarized during FDI contraction. This differential change in their membrane potential could then account for the difference in the effect of contraction on SICI and MEP/ICF.

Interestingly the data were similar in some respects to those reported in a previous study in human cortex which examined the effect of voluntary contraction on the response to low intensity 5 Hz rTMS. When given in the relaxed state, 5 Hz rTMS tends to decrease SICI without any effect on control MEPs (DiLazarro et al, 2002). Fujiwara et al (2004) found that the effect on SICI was enhanced during contraction of the target muscle (wrist flexors in their experiments), whereas it was reduced during contraction of the antagonists (wrist extensors in their experiments). A similar effect occurred in the present experiments using cTBS. On its own cTBS depresses SICI, and this effect was enhanced (prolonged) if cTBS was given during contraction of FDI. The effect of contraction on the response to iTBS has no clear parallel since SICI is usually increased by iTBS-relaxed whilst there was no affected on SICI if iTBS was applied during contraction.

6.4.2 Contraction immediately after TBS

Effects on MEP. Contraction immediately after cTBS had a very remarkable effect: it reversed the usual effect of cTBS from suppression to facilitation. It also prolonged the effect of iTBS. The results were not due to contraction alone since 1 min contraction had no lasting effect on MEPs. They were also unlikely to have been caused by the afferent feedback produced by muscle contraction since electrical stimulation of the ulnar nerve to mimic the voluntary contraction of FDI had no effect on the response to TBS. The conclusion is that voluntary activation of the motor cortex led to activity that interfered with the inhibitory after effects of TBS.

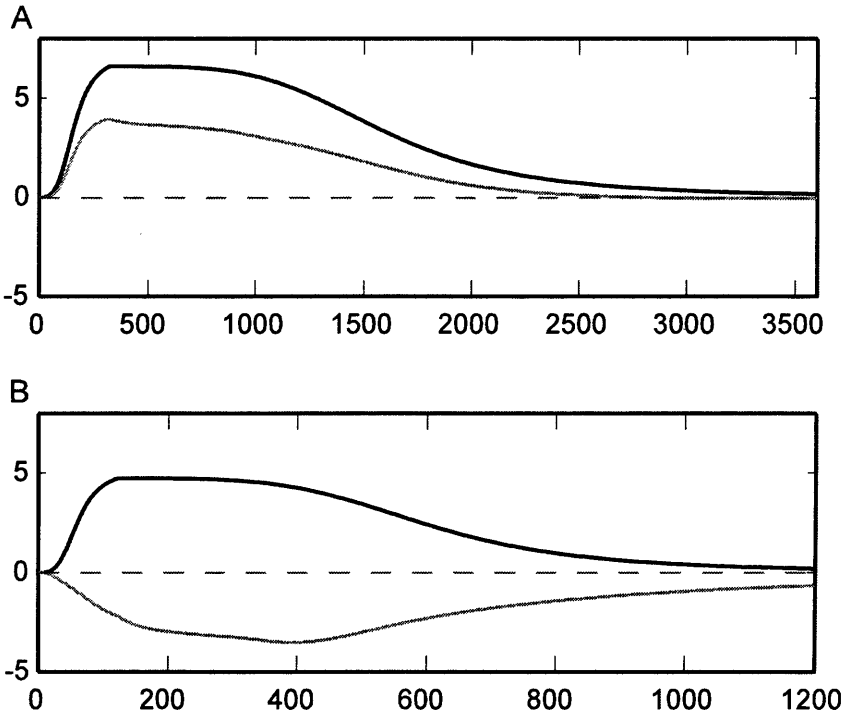

Fig 6.7 ***The simulated after-effect of TBS and TBS followed by contraction immediately.*** *Fig 6-7A shows the simulated after-effect of iTBS (grey line) was enhanced (black line) by subtracting the build-up of inhibitory effect. Fig 6-7B shows the simulated after-effect of cTBS (grey line) became opposite (black line) by subtracting the build-up of inhibitory effect*

It was a pleasant surprise to see that this could readily be accounted for by the model presented in Chapter 4. In that model, after-effects of both iTBS and cTBS are the result of a combination of underlying facilitatory and inhibitory effects. We found that if voluntary contraction abolished build up of inhibitory effects, then the resulting MEP changes reflected the time course of the facilitatory effect alone. This is shown diagrammatically in Fig 6.7. The consequence is that cTBS becomes facilitatory and the facilitatory effect of iTBS is enhanced.

Although there is no proof that this is occurring in the present experiments, it is not an unlikely scenario. For example, if our effect depends, as in the model on Ca^{2+} levels in the post-synaptic cell, then it is possible that activating the target muscle voluntarily may cause a sudden large influx of calcium into postsynaptic cells that desensitizes the inositol triphosphate ($InsP_3$) receptor (Bezprozvanny et al., 1991). Nishiyama et al (2000) showed that when $InsP_3$ receptor-dependent Ca^{2+} release from internal stores is blocked, an LTD protocol is reversed to produce LTP. On contrary, the facilitatory effect, which can be triggered by transient and large amount of Ca^{2+}-influx (Yang et al., 1999), is not affected by the blockage of $InsP_3$-induced Ca^{2+} release. Thus contraction could block the induction of LTD-like effects while preserving the LTP-like consequences of TBS.

Previous studies in animals have also shown that physiological activity after induction of LTP/LTD can reduce or abolish changes in synaptic plasticity. Interestingly, the effect is greatest, the nearer it is to the end of the induction period (Chen et al., 2001). The mechanism is thought to be similar to mechanisms of electrical de-potentiation/de-depression, and involve reversal of phosphorylation of glutamate receptors (Chen et al., 2001; Huang et al., 2001). However, unlike in the

present experiments, physiological activity in animals reduces LTP as well as LTD. One of the differences between animal studies and this study is that the event, including electrical stimulation, stress or stimulation by a novel environment that is needed for the modulation of LTP/LTD is a passive input, while it is a voluntary activation in our experiments. In addition, most events for reversal of LTP/LTD were not given immediately after plasticity induction and for so short a period as in the present experiments. It may be that this combination makes our own results differentiate between excitatory and inhibitory effects. If so then future experiments in humans will need to test how longer periods of contraction might influence TBS.

Effects on SICI. In contrast to the results on MEPs, the effect of cTBS on SICI was not reversed by the contraction. In fact, SICI was unchanged after cTBSc0. The most likely explanation for the difference is that during contraction, circuits involved in MEPs are likely to be excited whereas those involved in SICI onto the FDI muscle are likely to be suppressed. The difference in activation presumably affects the response to contraction, abolishing the effect of cTBS on SICI whilst it *reverses* its effect on MEPs.

6.4.3 Contraction at 10 min after the end of cTBS

In contrast to the effect immediately after cTBS, contraction after 10min, at a time when the inhibitory after-effect had built up towards maximum, had no permanent effect on MEP amplitude. There was a short-lasting reduction of suppression that might be related to post-exercise facilitation (Samii et al., 1996), but this lasted only a few minutes. This also is compatible with the model in Chapter 4 since at this time it assumes that all long term effects have built up to their final level. It also fits with

animal studies, which show that the reversal of LTP can only happen when a depotentiating event is applied within a short time window after induction of LTP (Xu et al., 1998; Huang et al., 1999; Manahan-Vaughan and Braunewell, 1999; Staubli and Scafidi, 1999; Chen et al., 2001). However, in those experiments, the time window for an effect was closer to one hour than 10 minutes. Perhaps if we had applied cTBS for longer, to produce a more lasting suppression of MEPs, then the time window for reversal by physiological activity may have been longer. Alternatively, it may be that if we had extended the duration of our contraction from 1 min to 5min, then a permanent effect could have been revealed even in the present experiments.

6.5 CONCLUSIONS

In conclusion, we have demonstrated that voluntary muscle contraction can interact with the long term effects of TBS on MEPs/SICI and ICF. One conclusion is that induction of presumed synaptic plasticity in the brain is likely to be optimal if synaptic activity is well controlled. It is also crucial to keep the target muscle relaxed in the period when the after-effects are building up, otherwise this may reverse any effects that might occur. However, once the plasticity effect has been well built up and has stabilised, it is reliable and is not easily abolished.

Chapter 7 Plasticity in carriers of the DYT1 gene mutation

This work *was an equally shared project with Dr Mark J Edwards*

The DYT1 gene mutation is a common cause of childhood-onset generalised dystonia (Ozelius et al., 1997; Bressman et al., 2000). However, its low age-dependent penetrance means that 60-70% of carriers never manifest symptoms (Bressman et al., 1994; Bressman et al., 2000). Since physiological and functional imaging studies have shown similar deficits in manifesting (M-DYT1) and non-manifesting (NM-DYT1) individuals (Eidelberg et al., 1998; Edwards et al., 2003), and since the abnormal gene product is produced in all carriers, it is not clear why NM-DYT1 carriers fail to develop clinical symptoms. Here we used transcranial magnetic stimulation (TMS) methods to assess mechanisms of neural plasticity in the motor cortex of healthy subjects, subjects with sporadic dystonia and in DYT1 carriers. We found increased synaptic plasticity in sporadic dystonia and M-DYT1 subjects, compatible with the suggestion that dystonic symptoms arise from an increased propensity to form associations between input and output of the motor cortex, leading to inappropriate combinations of muscle activity during voluntary movement. Importantly, synaptic plasticity in NM-DYT1 individuals was less responsive than normal. This may prevent them from developing unwanted synaptic connections and protect them from appearance of clinical symptoms. Understanding the mechanism of differential motor system plasticity in DYT1 gene carriers could lead to the identification of potential therapeutic targets capable of modulating the emergence of dystonia in at-risk individuals.

7.1 INTRODUCTION

Dystonia is a neurological movement disorder characterised clinically by sustained involuntary muscle contraction causing twisting and repetitive movements or abnormal postures (Fahn, 1986). Primary dystonia is defined as dystonia (with or without tremor) as the only disorder present, with no evidence of an underlying neurodegenerative process. This separates these patients from those with secondary dystonia, who have a clear secondary cause for the dystonia (for example a basal ganglia lesion, or a wider neurodegenerative process) and who often have symptoms in addition to dystonia such as seizures and cognitive impairment.

One common form of primary dystonia is due to a mutation in the DYT1 gene (Bressman et al., 1994; Ozelius et al., 1998), and these individuals present an intriguing phenomenon to the researcher with an interest in the pathophysiology of dystonia. This mutation, a single GAG deletion on chromosome 9q34 (Ozelius et al., 1997), is the commonest cause of young-onset primary generalised dystonia. However, only 30% of mutation carriers ever manifest dystonia, leaving the majority symptom free for life (Bressman et al., 1994). The manifestation of dystonia in these individuals is age dependent; carriers who pass the age of 30 without developing symptoms almost invariably remain free of dystonia. The natural phenomenon of manifesting (symptomatic) and non-manifesting (asymptomatic) DYT1 mutation carriers provides a unique opportunity to investigate the forces that drive the development of dystonia in genetically susceptible individuals.

Previous studies by ourselves (Edwards et al., 2003; Rothwell et al., 2003) and

others (Eidelberg et al., 1998; Eidelberg, 2003) have found surprisingly little difference between manifesting and non-manifesting mutation carriers, at least at a cortical level. Thus, all mutations carriers, regardless of whether they have symptoms or not, have a similar severity of abnormality in cortical motor inhibitory circuits, and a similar abnormal pattern of motor circuit activation on PET. We therefore hypothesised that affected gene carriers will have abnormalities in additional systems which allow dystonia to become clinically apparent. The age dependent nature of manifestation of dystonia in gene carriers suggests that there may be an important property of the developing brain that makes it more susceptible to the consequences of the DYT1 mutation. The system that underlies brain plasticity would be a strong candidate in this regard, as this property of the brain demonstrates an age-dependent decline in potency.

Several lines of evidence point to excessive/disordered neural plasticity in patients with dystonia. From a clinical standpoint, patients with primary focal limb dystonia frequently develop the condition following chronic performance of repetitive skilled movements of the affected body part – finger dystonia in musicians being a characteristic example (Byl, 2003). The task-specificity of dystonia in many individuals would also suggest a breakdown in the function of a particular, over-practised motor programmes (Frucht et al., 2001; Lim et al., 2001; Altenmuller, 2003). It is well known that the acquisition of motor and other skills relies on the ability of synapses to undergo changes in efficiency (Butefisch et al., 2000; Ziemann et al., 2001; Ziemann et al., 2004). In dystonia, neuronal representations of the dystonic body part have been found to be altered such that the highly specific somatotopic organisation of the sensory and motor cortices becomes degraded (Byl, 2003), and divisions between adjacent body parts blurred (Byrnes et al., 1998). This

blurring of divisions between adjacent areas has been characterised as a loss of "centre-surround" inhibition (Hallett, 2003). When a desired movement is selected, instead of adjacent areas being inhibited to maintain focus of the movement, there is a spread of activation resulting in unwanted muscle activity. One could hypothesise that in susceptible individuals, excessive synaptic plasticity occurring in motor areas in response to normal environmental stimuli, or overpractise of a particular movement pattern, might result in an overexcitable, unfocussed motor system producing unwanted muscle contraction when a particular movement is performed, or even at rest. The concept of an oversensitive system of brain plasticity is interesting with regard to DYT1 dystonia given the age dependent nature of penetrance in the condition.

In this current study, we hypothesised that brain, specifically the motor cortex, plasticity might drive the difference in clinical symptoms between manifesting and non-manifesting carriers of the DYT1 mutation.

7.2 METHODS AND SUBJECTS

7.2.1 Subjects

We recruited 8 DYT1 gene carriers with clinical dystonia (DYT1; mean age ± SD, 47 ± 8 years) from the movement disorder clinics at the National Hospital for Neurology and Neurosurgery, United Kingdom. Inclusion criteria were (1) genetic analysis positive for the typical DYT1 mutation; (2) onset of limb dystonia before the age of 25 years with or without subsequent progression; (3) no other cause for dystonia

revealed by investigation, including magnetic resonance imaging scans and blood tests; (4) no brain, spinal, or peripheral nerve surgery for dystonia or other cause in the past; (5) no history of other neurological disease; and (6) no use of botulinum toxin in the past 4 months. Subjects were permitted to continue their other medications as normal during the study. Six DYT1 gene carriers without manifesting clinical symptoms (NM-DYT1; mean age ± SD, 50 ± 8 years) were ascertained by genetic and clinical assessment of family members of the M-DYT1 group. Inclusion criteria were (1) genetic analysis positive for the typical DYT1 gene mutation; (2) clinical absence of dystonia confirmed by personal independent assessment of each patient by two neurologists; (3) no brain, spinal or peripheral nerve surgery for any cause in the past; (4) no history of neurological disease; and (5) age over 30 years. Eight patients with primary cervical dystonia were also recruited. Inclusion criteria were (1) dystonia localised in the cervical area without other body part involved; (2) no other cause for dystonia revealed by investigation, including magnetic resonance imaging scans and blood tests; (3) no brain, spinal, or peripheral nerve surgery for dystonia or other cause in the past; (4) no history of other neurological disease; and (5) no use of botulinum toxin in the past 4 months. Thirteen healthy age-matched controls were recruited from a departmental register of volunteers. The ethics committee of the National Hospital for Neurology and Neurosurgery granted ethical approval for the study. Subjects gave their written informed consent to participate.

7.2.2 Stimulation and recording

For all experiments, subjects were seated in a comfortable chair. EMGs were recorded using Ag-AgCl electrodes from the right first dorsal interosseous (the dominant hand in all subjects). EMG activity was recorded with a gain of 1000 and

5000 and filtered with a band-pass filter (3 Hz to 2k Hz) through Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK).

Magnetic stimulation was given using a hand-held figure of eight coil with an outer winding diameter of 70 mm (Magstim Co., Whitland, Dyfed, UK). Single and paired pulses were delivered by Magstim 200 machines, and rTMS was delivered using a Magstim rapid stimulator connected to four booster modules. Stimulation was delivered over the motor hand area with the coil placed tangentially to the scalp with the handle pointing posteriorly. The motor hand area was defined as the location on the scalp where magnetic stimulation produced the largest MEP from the contralateral first dorsal interosseous (FDI) when the subject was relaxed (the "motor hot-spot"). The stimulation intensity was defined in relation to the active motor threshold (AMT) of the subject. The AMT was defined for each Magstim machine separately as the minimum intensity of single pulse stimulation required to produce an MEP of greater than 200μV on more than five out of ten trials from the contralateral FDI while the subject was maintaining a voluntary contraction of about 20% of maximum in the FDI. Visual feedback was provided to the subject to help maintain a constant muscle contraction of the correct force.

7.2.3 Experiments

We explored the nature of motor system plasticity in dystonia using TBS. We hypothesised that patients with primary dystonia should have a more pronounced response to conditioning compared to normal subjects. If in contrast NM-DYT1 subjects were found to have a normal response to TBS, then this would be strong evidence that the system underlying brain plasticity is the one in which abnormalities

must occur in order for dystonia to become clinically apparent, and that conversely, normal function of this system can prevent the occurrence of dystonia in genetically susceptible individuals.

We assessed the time course of changes in MEP size elicited from the contralateral FDI by a single pulse of TMS delivered at a set intensity (the intensity required to produce an MEP of 1mV at rest before TBS) and short intracortical inhibition (SICI) using the paired-pulse method described by Kujirai et al (Kujirai et al., 1993) before and after conditioning with 20 seconds of continuous TBS (cTBS). We assessed SICI at ISI of 2 ms using a conditioning intensity of 80% AMT. We performed 10 trials of each condition (test MEP alone or paired pulse stimuli) given at variable intervals (4.5–5.5 s) in a random sequence in one block. Two blocks of baseline were recorded. After cTBS, one block was recorded immediately and every five minutes until 35 minutes after conditioning using the same intensity of stimulation used in the baseline assessment. In addition, we adjusted the intensity of the test stimuli while assessing SICI after cTBS to maintain the amplitude of test MEPs at approximately 1 mV in six age-matched control subjects to assess SICI every five minutes until 25 minutes..

7.2.4 Data analysis

Peak-to-peak amplitude was measured as MEP size. Mean MEP size was calculated for each subject for the baseline and for each five minute interval after rTMS. A two-way analysis of variance (ANOVA) with GROUP (patients vs. controls) and TIME (before vs. after rTMS) as main factors was performed to compare different groups. Repeated measures ANOVA was also used to compare variables before and

after TBS. Statistics were performed using *SPSS for Windows v. 11.0.*

7.3 RESULTS

All subjects completed the experiments and none reported any lasting side effects.

7.3.1 MEPs

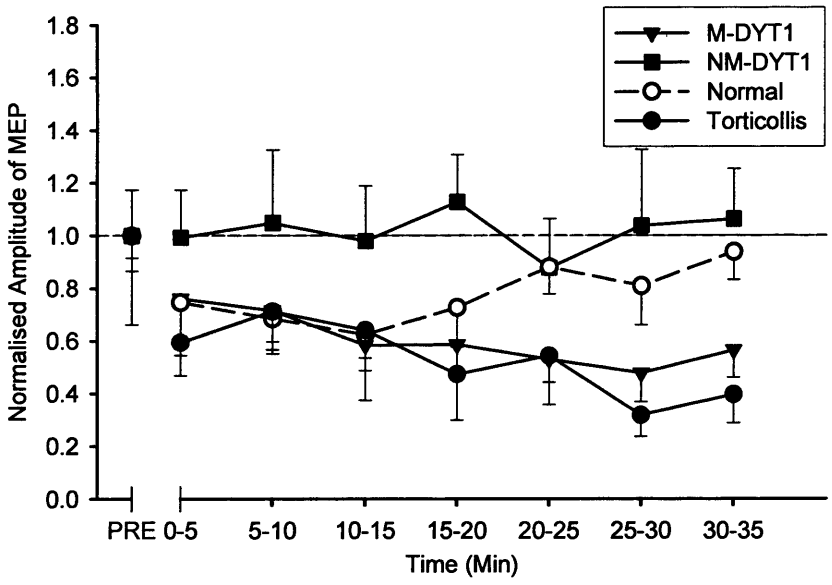

Fig 7.1 ***Normalised amplitude of MEP following cTBS in M-DYT1, NM-DYT1, torticollis and control subjects.*** *Control subjects showed a significant reduction in MEP size lasting about 20 minutes following cTBS. Both M-DYT1 and torticollis subjects showed a significantly longer suppression of MEP size compared to normals and NM-DYT1 subjects. NM-DYT1 subjects showed no significant change in MEP size at any time point following cTBS.*

A two factor ANOVA with TIME and GROUP (i.e. normal, torticollis, M-DYT1 NM-DYT1) as main factors revealed a significant TIME x GROUP interaction

($F(21,154)=2.73$, $p<0.001$). (Fig 7.1) Patients with torticollis and M-DYT1 showed a significantly longer response to conditioning compared to normal subjects (TIME x GROUP: $F(7,77)=3.84$, $p=0.001$, $F(7,84)=2.99$, $p<0.01$, respectively) with MEPs still maximally suppressed at 35 minutes after conditioning ($F(7,35)=4.28$, $p<0.005$, $F(7,42)=5.00$, $p<0.001$, respectively), while MEPs was only suppressed for around 20 minutes in normal subjects ($F(7,42)=3.51$, $p=0.005$). In direct contrast to this, NM-DYT1 subjects showed no significant change in MEP size at all at any time point after conditioning ($F(7,35)=0.61$, *ns*), significantly different in this respect from both normal (TIME x GROUP: $F=2.23$, $p<0.05$) and dystonic subjects (NM-DYT1 vs. M-DYT1: $F=2.90$, $p<0.05$; NM-DYT1 vs. torticollis: $F=3.70$, $p<0.05$).

7.3.2 SICI

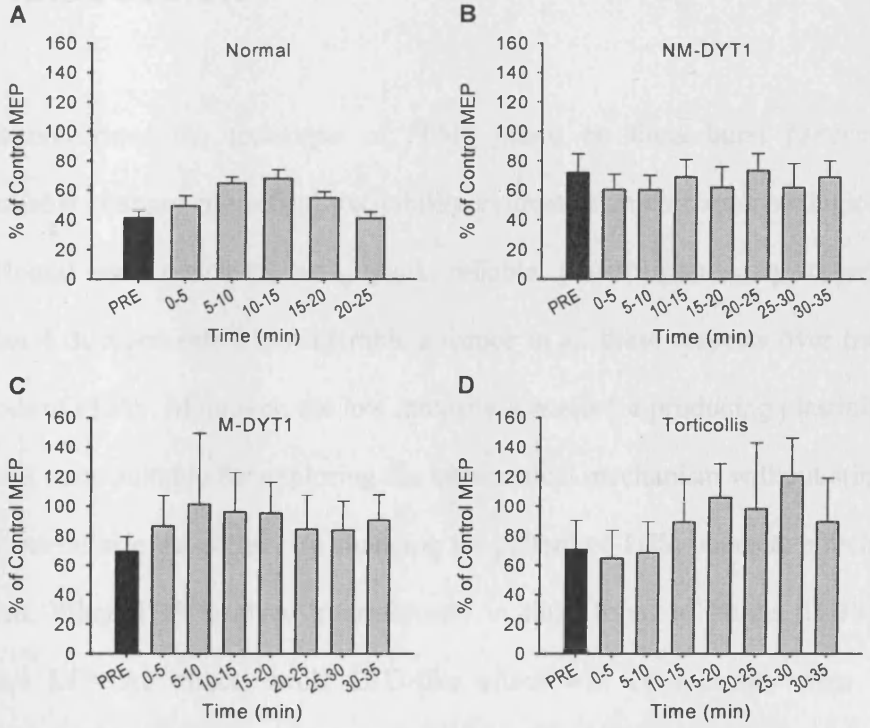

Fig 7.2 ***SICI following cTBS in M-DYT1, NM-DYT1, torticollis and control subjects.*** *(A) shows the results of control subjects. The test intensity was adjusted to maintain MEP size to be 1mV following cTBS. The SICI was significantly decreased by cTBS until 20 min. (B) The SICI did not change after cTBS conditioning. Both M-DYT1 (C) and torticollis (D) subjects showed a tendency of decrease of SICI while MEPs were suppressed.*

Fig 7.2 shows the data for SICI following cTBS in normals and patients. The test intensity was adjusted to maintain the size of test MEPs to be around 1mV after cTBS conditioning, while it was not adjusted in patients. The amount of SICI tends to be reduced by cTBS in M-DYT1 and torticollis patients. However, because the MEP sizes changed after cTBS we can only comment on the NM-DYT1 group. A two factor ANOVA with TIME and GROUP (i.e. normal with test intensity adjusted after conditioning and NM-DYT1) as main factors revealed significant main effect of a GROUP x TIME interaction (F=2.90, p<0.05). The amount of SICI was reduced by cTBS for up to 20 min (F(5,25)=5.41, p<0.005) in normal subjects, but was not changed in patients of NM-DYT1 (F(5,25)=0.64, *ns*).

7.4 DISCUSSION

We demonstrated the technique of rTMS based on theta burst patterns offers controllable changes in cortical excitability evident at an electrophysiological and a behavioural level which are safe, quick, reliable, powerful and easy to produce in Chapter 4. It represents a considerable advance in all these respects over traditional methods of rTMS. Moreover, the low intensity it needs for producing plasticity effect makes it more suitable for exploring the intracortical mechanism without stimulating structures outside the cortex. By changing the pattern of TBS, opposite effects can be induced. When TBS is given intermittently in short trains of bursts (iTBS) it will produce LTP-like effect, while LTD-like effect will be induced when TBS is delivered continuously (cTBS).

In the experiments of this chapter, we have further demonstrated that the effect

of TBS is reproducible. The MEPs of healthy controls were suppressed by 20 seconds of cTBS for up to 20 minutes, which is consistent with the results shown in Chapter 4. In addition, cTBS showed more powerful suppression effect on the MEPs in patients with M-DYT1 and primary cervical dystonia. The suppression effect outlasted the conditioning by more than 35 minutes and showed no tendency of returning to the baseline at the point we stopped recording. On contrary, the amplitude of MEPs was not modified by cTBS in NM-DYT1 individuals at all.

Not only the size of MEP, but also the amount of SICI, can be decreased by cTBS conditioning in healthy subjects. In this study we also tested SICI in all subjects. However, we did not manage to ask patients to come back to repeat the experiment with matched MEP sizes. Therefore, in patients with clinical symptoms who's MEPs were suppressed, the SICI data may be unreliable due to changed MEPs size. Even though, the amount of SICI still tended to be decreased in these patients. As regards of NM-DYT1 individuals, since their MEPs were not changed by cTBS, their SICI data would reflect what they should be. Identical to the results of MEPs, the SICI of NM-DYT1 was resistant to cTBS conditioning.

These data place abnormalities in motor system plasticity at the heart of a theory to explain dystonia pathogenesis. We have demonstrated that individuals with genetic and non-genetic primary dystonia have an exaggerated response to an experimental "plastic force". In support of the hypothesis of oversensitive neuronal plasticity in dystonia, previous studies have found that experimental interventions capable of producing temporary plastic changes in the motor/sensorimotor system in normal subjects (e.g. paired associative stimulation (PAS) and repetitive transcranial magnetic stimulation (rTMS)) can produce significantly more intense changes in

focal dystonic subjects (Quartarone et al., 2003; Siebner et al., 2003). Quartarone and colleagues (2003) suggested that increased PAS might predispose the motor system to form extra or unwanted connections between inputs and outputs that lead to overflow of muscle activity when movements are performed. The present study used a repetitive stimulus paradigm rather than a paired paradigm to test synaptic plasticity and came to a similar conclusion, at least in the M-DYT1 patients. In fact, excess response to a "plastic input" might also be the explanation of why we had previously found that the third phase of spinal reciprocal inhibition could be affected by rTMS to the motor cortex in M-DYT1 patients whereas there was no change in healthy subjects (Huang et al., 2004). Perhaps subcortical as well as cortical systems show similar abnormalities in synaptic plasticity. The decrease of GABAergic activity appearing in patients with dystonia was used to explain the increased facilitatory effect of PAS in writer's cramp patients (Quartarone et al., 2003).

However, NM-DYT1 individuals who are symptom-free despite their genetic susceptibility to dystonia, and who have abnormalities in cortical GABAergic inhibitory circuits of a similar severity to M-DYT1 individuals (Edwards et al., 2003), lack the increased "plastic" response of manifesting patients. Indeed they show *less* "plasticity" than normals in that their response to the probe sequence was underactive. This combination is compatible with the idea that not only is the increased response of the motor cortex to the "plastic" probe sequence linked to appearance of dystonic symptoms, but also that underactivity of this mechanism may protect susceptible individuals from appearance of dystonic symptoms. If this is correct, the reduced sensitivity of the system underlying plastic change in the brain may have implications for other brain functions. Indeed, it has recently been reported that there is a deficit in the ability to perform motor sequence learning in NM-DYT1

mutation carriers (Ghilardi et al., 2003). We would also predict that prior to appearance of clinical symptoms, motor cortex plasticity might be increased in those DYT1 carriers who are to develop dystonia, whereas it would be reduced in those who will not manifest symptoms, potentially allowing the pre-symptomatic identification of those mutation carriers who are likely to develop dystonia. Future studies to identify the mechanism of differential plasticity in DYT1 carriers could determine potential therapeutic targets capable of being exploited to modulate the emergence of clinical dystonia in susceptible individuals.

7.5 CONCLUSIONS

In gene carriers who develop dystonia, there are additional abnormalities in their response to an experimental plastic force, with an exaggerated response compared to normal subjects. This excessive plasticity would fit well with clinical and experimental evidence from patients with dystonia, and appears to be a fundamental feature of the pathophysiology of dystonia. In contrary, NM-DYT1 individuals show a resistance to an experimental plastic force. This confirms the central importance of motor system plasticity in the genesis of dystonia, and provides an explanation as to the ability of these genetically susceptible individuals to remain symptom free.

The excessive response of dystonic individuals to experimental interventions that produce plastic changes in the brain means that it might be possible to create plastic changes that could be of therapeutic benefit. Unfortunately, traditional methods of rTMS, while capable of producing plastic changes evident at an electrophysiological level, have been disappointing when used to produce

behavioural changes in the motor system. We have developed novel paradigms of rTMS based on theta burst patterns that are able to produce controllable and long-lasting changes in motor system function at an electrophysiological and behavioural level that are quick, safe and powerful. This type of rTMS has potential as an experimental and therapeutic tool.

Chapter 8 Discussion

In the thesis, I have demonstrated that rTMS given in bursts at high frequency and low intensity is capable of producing consistent and controllable electrophysiological and behavioural changes in the function of the human motor system. These paradigms appear to be safe, and effects are seen after only seconds or a few minutes of rTMS conditioning, which is much quicker than other non-invasive methods of inducing long term changes in cortical excitability in conscious humans. In particular, I have found that the pattern of delivery of TBS (continuous versus intermittent) is crucial in determining the direction of change in cortical excitability. In the thesis I interpreted these changes in excitability and behaviour as being caused by changes in transmission at cortical synapses, and refer to them as "cortical plasticity".

I then developed a mathematical model to try to understand why the pattern of stimulation is crucial in determining the direction of plasticity. This model is not only useful for explaining the importance of the pattern of stimulation, but also provides a hypothesis that can be used to devise further experiments into the mechanism of plasticity induced by repetitive stimulation on animals or humans. Moreover, by asking subjects to activate the target muscle voluntarily during or even immediately after the conditioning, I demonstrated that the status of motor cortical excitability is capable of modulating the effect of plasticity induced by TBS. Finally, I demonstrated an abnormal response to TBS in patients with primary dystonia and in sub-clinical DYT1 carriers using TBS. The excessive plasticity found in patients with dystonia would fit well with clinical and experimental evidence from patients with dystonia, and may be a fundamental feature of the pathophysiology of dystonia. The gene carriers who do not develop dystonia show a resistance to TBS-induced plasticity, indicating that this might be part of some protective mechanism that

prevents the development of dystonia.

These findings have implications for both the use of rTMS in the study of human motor physiology, and in the use of rTMS in the treatment of disorders of motor plasticity. Traditional methods of delivering rTMS require lengthy periods of conditioning to produce lasting effects, and the stimulus intensities necessary to do so can be uncomfortable for the subject, and technically difficult due to coil overheating in subjects with high motor thresholds. The effects of such stimulation have been found to have a high inter-individual variability, and to produce weak effects, in particular on a behavioural level. The results of studies using traditional methods of rTMS to treat disorders such as Parkinson's disease and dystonia have so far been relatively disappointing. The method of stimulation presented here appears to provide a powerful, controllable and consistent effect on the motor system with very brief periods of conditioning delivered at a low intensity. This TBS-style stimulation might have additional advantages in therapeutic applications both in terms of conditioning time to effect time ratio, the consistency of the effect, and the ease of giving repeated sessions of stimulation.

However, I still lack direct evidence for the mechanism responsible for the effects of TBS. The fact that (i) TBS produces no change in spinal H-reflexes, yet (ii) modulates SICI/ICF is strong evidence that the effect occurs because of changes in excitability of neuronal circuits in the cerebral cortex. However, whether it involves plasticity at synapses, or is the result of long term changes in function of ion channels in neural membranes is unknown. In the thesis I have speculated that synaptic plasticity may be involved, but to obtain further proof for this would need further experiments. For example, it would be useful to perform pharmacological

experiments using drugs related to receptors affecting synaptic transmission, e.g. NMDA, glutamate or voltage-gated channel blockers to verify the synaptic location of TBS phenomena. Nitsche et al (2004) has used D-Cycloserine, a NMDA agonist, to show that both anodal tDCS, which enhances motor cortical excitability, and cathodal tDCS, which diminishes excitability, seem to depend on NMDA receptors. These kinds of receptor-related drugs were also used to test the mechanism of use-dependent plasticity (Ziemann et al., 1998; Butefisch et al., 2000). It would be particularly interesting, for example, to apply a low potency NMDA receptor blocker (e.g. dextromethorphan) or a voltage-gated Na^+ and Ca^{2+} channel blocker (e.g. lamotrigine) to decrease Ca^{2+}-influx during TBS conditioning. In this case, the effect of iTBS may reverse to depression, while the effect of cTBS remains depressive. This would demonstrate that postsynaptic Ca2+-influx is crucial in TBS as it for synaptic plasticity in many animal studies.

The question of the nature of TBS effects could also be tackled directly in animal studies. However, such experiments are not easy, especially on small animals, since the size of the skull and available coils limit the currents that can be induced in the brain. Certainly, stimulation will be much less focal in relation to the size of cortical areas than in humans, and the distribution of current may be quite different. In addition, animals are usually anesthetised during application of TMS and anaesthesia itself is well known to have effects on the brain's responses to TMS. Despite these problems, a small number of animal studies have used rTMS successfully in recent years. For example, Wang and colleagues (1996) demonstrated frequency-dependent LTP-like and more durable LTD-like changes in evoked spike rate using rTMS at a frequency of 1 to 10 Hz in rodent auditory cortex. Moreover, synaptic strength was enhanced by 10 1-s trains of 25 Hz rTMS at a low but not high

intensity in the rat hippocampus (Ogiue-Ikeda et al., 2003). It would be useful to probe the effects of TBS in the same models and if successful to use more invasive pharmacological and physiological methods to examine the nature of the effect in more detail. It may even be possible to demonstrate a similar pattern of effects with electrical stimulation of animal brain slices. This would be helpful in understanding the mechanism of the pattern-dependent effect and in proving the time course of excitatory and inhibitory effect that I have assumed in the model.

Turning again to human experiments, it would be very useful to explore the action of TBS on other cortical areas in the future. I will probably start by studying the premotor or prefrontal cortex, since both areas have already been used with conventional rTMS methods with some success. Subthreshold 1 Hz rTMS of the premotor cortex induces an inhibitory after-effect on corticospinal excitability in the ipsilateral motor cortex, whereas the same stimulus applied to motor cortex has no effect (Gerschlager et al., 2001; Munchau et al., 2002). The lower threshold of premotor stimulation might be because it is easier to stimulate the premotor cortex, which is located on the crown of the precentral gyrus, than the primary motor cortex, which is located in the anterior bank of the central sulcus. Experiments on more frontal areas are of potential therapeutic interest since many studies have claimed that rTMS to these area can have beneficial therapeutic effects on psychiatric problems (Pascual-Leone et al., 1996; Padberg et al., 1999; George et al., 2000; Burt et al., 2002). If TBS were a more effective method of conditioning the cortex, it would have the advantage of speed over conventional 1 Hz rTMS.

It is also important to see how TBS to one area influences connectivity with other areas, or even between areas at a distance. Neuroimaging, e.g. fMRI, and MEG

or EEG experiments would be a useful way to probe these questions. A recent [^{18}F]FDG-PET study showed increased glucose metabolism of bilateral primary motor areas and left SMA after 5-Hz rTMS at subthreshold intensity was given over left primary motor area, demonstrating the remote effect of 5-Hz rTMS over primary motor area on other motor areas (Siebner et al., 2000). On contrary, 1-Hz rTMS over left primary motor cortex was shown to cause widespread bilateral decreases lasting for at least one hour in prefrontal, premotor, primary motor cortex and left putamen by measuring regional cerebral blood flow in a $H_2{}^{15}O$-PET (Siebner et al., 2003). A functional MRI (fMRI) study (Lee et al., 2003) confirmed the widespread changes induced by subthreshold 1-Hz rTMS over primary motor area, and also showed that this was accompanied by changes in task related activity in contralateral premotor cortex and adjacent areas of ipsilateral sensorimotor cortex. In addition, endogenous dopamine release in ipsilateral caudate nucleus induced by 10-Hz rTMS over prefrontal (Strafella et al., 2001) and in ipsilateral putamen by 10-Hz or 5-Hz rTMS over primary motor area (Strafella et al., 2003; Ohnishi et al., 2004) have been demonstrated by [^{11}C] raclopride PET. I shall give subjects TBS and scan their brain in an optimal time window (i.e. after the effect is stabilised and before the effect disappears) and compare it with the result of the sham session. Alternatively there is the possibility of giving rTMS in the scanner, but for TBS, it maybe limited by the pattern of stimulation.

From a potential therapeutic point of view, it is important to test how plasticity induced by TBS interacts with behaviour in healthy subjects as well as in patients with neurological disease, especially if we have more detailed information on the precise mechanism of action of TBS. We have demonstrated in Chapter 7 that excessive plasticity may be a fundamental feature of the pathophysiology of dystonia.

This fits well with the idea that the excess muscle activity that characterizes dystonia is the result of the formation of extra, unwanted synaptic connections in the motor system that recruits more activity than necessary when a movement is performed. It would be also interesting to test motor plasticity in Parkinson's disease, which has been shown to have reduced striatal plasticity due to lack of dopamine stimulation (Calabresi et al., 1997) and to have abnormal bidirectional plasticity in animal models of dyskinesia (Picconi et al., 2003). I shall test whether iTBS and cTBS could reverse the effect of each other, and how these interactions affect behaviours in conscious humans.

It would be useful to see if the mechanism probed by TBS is the same as that probed by other methods of inducing cortical plasticity in humans, such as rTMS, tDCS or PAS. I speculate that rTMS and tDCS may have different mechanisms, because TMS may work on neurones, while tDCS may work by changing the membrane potential of (the same?) neurones. TBS may even work differently from traditional regular rTMS because of its low intensity and high frequency. PAS involves a different time dependent associative plasticity and is therefore very likely to be different from TBS. The most direct way to test for any shared effects is to use TBS before or after one of the other paradigms to see whether it is possible to alter the effect of each given alone. The interaction between the results may give us some clues about whether they share similar mechanisms.

Finally, I would like to see whether it is possible to improve the parameters for TBS, especially those that lead to facilitation. According to the model, a burst with more pulses, a burst at higher frequency and a shorter inter-train interval are all potential candidates.

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